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Levy, Detwiler, and Copenhaver (p. 659) found numerous oral developmental anomalies in *Amblystoma punctatum* which had been subjected to low oxygen tension or to a synthetic growth hormone while in embryonic state. The effects may have resulted from suppression of developmental rates of the mesoderm or ectoderm.

Bradford (p. 663) investigated the structure of the teeth of a fish (*Pristis microdon*) which grew from persistent pulps. These rostral teeth were composed of a dentin of rod form, more similar to enamel than to dentin of other teeth. They are of further interest for the "roots" appear to increase in size by peripheral deposit of the rod-form dentin.

Miller's Novice Award winning paper of 1956 is presented on p. 669. The blood and nerve supply of the mandible was interrupted in experimental animals to determine the effect on deciduous tooth resorption and permanent tooth eruption. Resorption appeared to be related directly to vascularity but very early in life (7 days in rabbits) resorption was well enough advanced that osteoclastic activity had little effect on exfoliation. While eruption rates of rat's incisors did not prove to be sensitive to changes in blood supply, resection of the inferior nerve in rats accelerated eruption rates. It is explained that the latter phenomenon may be related to continuous eruption and attrition rather than to direct effects.

Trypan blue injected into pregnant rats has produced a high percentage of congenital anomalies. Kreshover, Knighton, and Hancock (p. 677) applied this method in an attempt to produce congenital dental defects in rats and mice. Gross malformations were produced but dental defects were not observed. Why the dental apparatus is spared from the toxic effects of trypan blue is not apparent.

Burnett and Zenewitz (p. 684), stimulated by an earlier finding that the heat-labile portion of the teeth of hamsters is greater than that in the teeth of other rodents and human subjects, used a KOH-ethylene glycol method to investigate this problem further. Although the extractable fraction of the hamsters' teeth contained some organic material, the amount obtained was less than that removed by heat (900° C). The calcium-phosphorus ratios differed between the two methods. The fallacy of comparing ratios obtained from different species by different methods is evident.

Weisenstein and Green (p. 690) studied the oral flora and dental conditions of 89 adults who never had developed any carious lesions, i.e., immunes. The lactobacillus counts were zero or low with only smooth colonies (the "immune pattern"). In 5 individuals who developed their first carious lesions while under study the bacteriologic pattern was different. This group of immunes is the basis for a broad study of dental caries.

Rogosa, Johansen, Disraely and Beaman (p. 695) studied the oral and fecal flora of the hamster in relationship to the progression of dental caries. Johansen's technic was modified to permit this investigation of bacteria from the living animal with simultaneous caries examinations. Streptococci and lactobacilli, although they are minority members of the salivary flora, were singled out for quantitative studies. Salivary streptococci were not related to caries progression in either sex and it was only in the male, and after extensive progression of lesions, that salivary lactobacilli showed a positive relationship with dental caries. The fecal flora was not comparable to the oral flora in the relative numbers of streptococci and lactobacilli. This, the authors conclude, does not support the concept that coprophagy is important in establishing or maintaining the oral flora. The authors recognize that studies of the salivary flora need not reflect the total oral flora. It appears that technics should be developed for comparable quantitation of the flora of the plaque, both in experimental animals and in man. The salivary flora may not reflect the oral flora at any given time any more than the fecal flora reflected the flora of the cecum. As difficult as studies of the oral flora may be they are essential for a clearer understanding of health and disease.

Bixler, Muhler, and Shafer (p. 709) were unable to establish the salivary glands as intermediates in the sex hormone-dental caries relationship in the rat, although they previously had suggested that the thyroid-dental caries relationship in these animals is activated through salivary glands. The hormonal influence on caries in the rat is interesting

and the findings from Indiana suggest that the influences may operate through different pathways.

Chauncey, Lionetti and Lisanti (p. 713) studied the esterases of parotid saliva. Nonspecific esterases, cholinesterase, and lipase of saliva attacked a β naphthylacetate. The esterase activity of parotid saliva shows similarities to that of serum esterase but also exhibits differences. Certain standards for the salivary enzymes were established.

Because of the suggestion that collagenase may be active in periodontal disease, Swanson (p. 717) analyzed, for the enzyme, saliva from 30 individuals with known active periodontal inflammations. Since none was identified it was concluded that, if present, its concentration is too small to be active in the disease or that it may be bacterial in origin and concentrated in small regions.

Kerr, Ramfjord, and Grape-Ramfjord (p. 721) exposed animals to the powder used in the airbrasive technic. The animals received high concentrations of the powder by confinement in air-tight containers in which the powder was agitated. The powder produced adverse pulmonary changes in monkeys and rodents but, due to the low concentration of dust in the dental office and the periodic use of the technic, it is not considered a health hazard to dentist or patients. The authors do recommend the elimination of the silica and elimination of all particles smaller than 3 to 5 microns.

The microhardness of enamel surfaces is of great importance in its relationship to dental disease, resistance to caries and effect of hardening agents. Caldwell, Muntz, Gilmore, and Pigman (p. 732) measured the surface hardness of teeth. Deviations in hardness on a single surface of a given tooth were often as great as differences between different teeth and different tooth surfaces. The hardness of polished tooth surface was less than that of intact surface but more constant from area to area. The authors' results suggest the need for careful re-evaluation of the effect of such agents as fluorides on surface hardness although hardness and solubility are not synonymous.

Because of the difficulty of measuring the hardness of the teeth of small animals, Taketa, Perdue, O'Rourke, Sievert, and Phillips (p. 739) devised an abrasive method for measuring wear resistance of these teeth. They describe the apparatus and analyze its variables.

Geller and Rovelstad (p. 745) analyzed saliva by spectrophotometry. They found peaks that could be duplicated by 0.1 per cent protein (albumin), 0.05 per cent nucleoprotein (yeast), 0.05 per cent hemoglobin and 0.5 mg. per cent uric acid. Further studies by this method may offer data that are difficult to obtain from this mixture of secretion and oral contents.

Landing, Lahey, Schubert, and Spinanger (p. 750) found a large increase in the iron content of teeth from a child with tranfusional hemosiderosis. These findings should be considered with Isaac and Brudevold's paper (p. 753).

Isaac and Brudevold (p. 753) exposed tooth surfaces to 1,000 and 100 ppm of salts of copper, iron, tin, silver, mercury, and lead for 15 minutes and then exposed to hydrogen sulfide for a similar period. Discolorations were noted. After the surfaces were brushed and re-exposed to hydrogen peroxide lesser staining was observed. Tin, mercury, iron, and silver salts produced the heaviest and most persistent stains. Since many foods contain these salts and since hydrogen sulfide is available in the mouth, their possible role in tooth darkening with age is discussed. The use of these metals in caries-control methods is also considered.

Mardfin and James (p. 759) studied the relative effect of chelation and nitric acid demineralization on the staining of formalin-fixed tissues by histochemical methods. While the chelated tissues stained adequately, it is suggested that further studies be instituted to determine optimal conditions.

Coolidge and Jacobs (p. 765) carefully compared normal enamel and enamel from early carious lesions to determine what chemical factor is lost at early stages of the carious process. Carbonate is lost faster than calcium and phosphate in the early phases of caries.

Neuwirth and Baerger (p. 769) used the chronogram method to identify the acids produced when saliva is incubated with glucose. Lactic and pyruvic acids were produced in greatest quantity during the first 30 minutes with malic, succinic, and fumaric acids being found constantly and alpha-ketoglutaric acid and oxalacetic acid in most samples.

Using radioactive fluoride and a Geiger tube counting method, Brudevold, Hein, Bonner, Nevin, Bibby, and Hodge (p. 771) studied the reaction of the enamel surface to 1 ppm of sodium fluoride solution. The fluoride was taken up rapidly (93 per cent on the

first 9 minutes) but brushing with water caused a marked loss during the first 3 minutes. Pumiced, ground, etched or demineralized surfaces had greater uptake than intact surfaces. It is suggested that the penetration is very limited and that the acquisition of fluoride by enamel is chiefly a surface phenomenon. Since pumicing resulted in greater uptake, but also in loss after fluoridating, the Knudson technic of pumicing before the first topical application of fluoride, but not before the next three, appears sound. Perhaps, in view of the 9-minute result, one 10-minute application, during which time the tooth is kept moist, should be evaluated.

While stannous fluoride solution appeared to be more effective as a carious control agent than sodium fluoride when applied as topical application, stannous fluoride is extremely unstable. Gish, Muhler, and Howell (p. 780) tested a 4 per cent solution of potassium fluorostannite, which has the advantage of being stable, on 894 children. Marked reduction of caries (54 per cent by DMFT, 39 per cent by DMFS) was observed against distilled water controls. Topical applications will remain important for children not consuming commercial waters or living in areas where ignorance, prejudice, or apathy prevent fluoridation.

Gish, Howell, and Muhler (p. 784) report a 21 (DMFT) or 26 (DMFS) per cent greater reduction in new carious lesions when one application of 8 per cent stannous fluoride was used in comparison with 2 per cent sodium fluoride. The disadvantage of stannous fluoride lies in its instability but the use of other tin-fluoride compounds (potassium fluorostannite, p. 780) may be feasible. Longer application time (Brudevold, Hein, Bonner, Nevin, Bibby, and Hodge, p. 771) may also present advantages.

The effect of fluorides on dental caries activity has led to the investigation of the cariostatic effect of various fluoride compounds and other compounds. Muhler (p. 787) reports the results of administering possible caries inhibiting compounds to rats in drinking water. Caries rates were significantly reduced by stannous fluoride, potassium fluorostannite, and by stannous chloride and stannous gluconate with added sodium fluoride. Stannic fluoride and sodium fluoride were effective at a lower level of confidence while vanadium pentoxide, ammonium silicofluoride, sodium hexafluorostannite, and potassium fluoride were ineffective. Stannous salts without fluoride did not reduce caries. Continued studies of this type may eventually develop a pattern which will give a clue to why any given compound can reduce caries significantly.

Zwaardemaker and Tromp (p. 795), the former a medical health officer for 3 Dutch municipalities, and the latter a geophysicist with long experience in medical geography, combined their talents to present the geographical distribution of caries in young school children of the Netherlands. This interesting preliminary report, terminated by the health of the senior author, shows a great variation in caries' activity in various areas among a rather small and stable population.

The Proceedings of the International Association for Dental Research (p. 800), again this year, include only the Executive Proceedings and related matters. The abstracts appeared in preprinted form and were available before the meeting. Additional copies may be ordered from the Secretary, Dr. Dan Y. Burrill, 311 E. Chicago Ave., Chicago, Ill.

The British Division (p. 805) heard a total of 33 papers at its Fifth Annual Meeting in London on April 11 and 12, 1957. It is interesting to note that a greater percentage of the papers presented in London dealt with morphologic studies in comparison with those heard at Atlantic City the previous month. The quality of the papers, as judged by the abstracts, appears to be quite high. These abstracts offer the reader an opportunity to learn the results of research in capsule form and to familiarize himself with the scope of dental research in England.

A comment on Wagner and Muhler's paper in the August issue (p. 552), given in the Editor's Viewpoint (p. 494) inadvertently stated that differences in retention of fluorides from "naturally" and "artificially" fluoridated water may have been due to interference with intestinal absorption by *organic* components of naturally fluoridated waters. This should read *inorganic* components.

Feldman, Morken, and Hodge (April issue, p. 192) have indicated that calcium or magnesium may bind as much as 2.8 and 28 per cent, respectively.

H. B. G. R.

William John Gies
1872-1956

To dedicate an issue or a volume of the JOURNAL OF DENTAL RESEARCH to its founder, Dr. William J. Gies, would be redundant. The entire JOURNAL, since its founding in 1919, has served as a tribute to him. Rather, we feel it in order that the Resolution of Tribute to Dr. Gies, adopted by the International Association for Dental Research, should be reproduced here as a record of the respect of the Association members.

Dr. William John Gies died on May 20, 1956, in his 85th year. His life was full of accomplishment and dedicated to improving the lot of mankind. The members of the International Association for Dental Research wish to express their deep sorrow and extend their sympathy to his family and personal friends.

Dr. Gies' notable achievements in the advancement of biochemistry at the turn of the century were a prologue to the fundamental contributions he made to the advancement of dentistry. Never one to avoid a difficult problem, he undertook the task of improving the status of dentistry. He evaluated the profession and its needs in the famous *Bulletin on Dentistry* published in 1926. This report served as a blue print for the building of a scientifically oriented dental profession. Transforming vision into reality he used his skill in the management of men to establish our Association and its Journal. The unprecedented growth of the Association and its Journal is ample testimony for the soundness of his judgment and his single minded devotion. For his more than forty years of effort in behalf of dentistry we owe him an incalculable debt.

Few organizations have been more closely identified with a single man and none could have been more fortunately identified. As long as our Association and Journal endure, they will be a living and most appropriate memorial to our founder, Dr. William J. Gies.

Respectfully submitted,

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THE PRODUCTION OF DEVELOPMENTAL ABNORMALITIES OF THE ORAL STRUCTURES IN AMBLYSTOMA PUNCTATUM

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IN SEVERAL publications, Detwiler and Copenhaver[1, 2] and Copenhaver and Detwiler[3] described the results of subjecting the early embryos of *Amblystoma punctatum* to changes in the physicochemical environment. Their experiments were done primarily to ascertain whether, in the Urodeles, suppression of development at "critical" stages would bring about head duplication or twins such as Stockard[4] obtained with fish embryos (Fundulus and trout) when subjected to atmospheres of low oxygen or low temperatures. Detwiler and Copenhaver obtained striking arrests in the developmental rate by growing pregastrula, gastrula, and postgastrula embryos in atmospheres of low oxygen tension (1 to 0.05 per cent), high CO_2 tensions (17 to 40 per cent), and in various concentrations of indolebutyric acid (one of the synthetic plant growth hormones). Regardless of the duration of exposure to the suppressing agent or the stage of development of the embryo, not a single case of twinning was produced in over 2,000 experiments. These studies brought into question the general applicability of Stockard's theory that duplications in nature are the result of retardation of the developmental rate at critical stages of development.

With the use of the suppressing agents indicated above, Detwiler and Copenhaver obtained a variety of developmental abnormalities in a low percentage of cases. These were listed as follows: suppression of the bilaterality of the hemispheres (telencephalon media), cyclopia completa or incompleta, fusion of the olfactory organs, malformation of the vascular system including rudimentary bilateral hearts, delayed establishment of the circulation, duplication of balancers, and a partial suppression of the segmentation of the mesoderm.

Despite the main syndrome of head abnormalities which they obtained, the authors did not make any observations on the development of the jaws and teeth in the reported studies. Now, because of an interest in fundamental embryologic problems concerned with oral development, we restudied the sections of critical cases of the jaws and teeth in those embryos which showed other abnormalities. This communication deals, therefore, with various types of oral abnormalities which occurred in the embryos under consideration.

Received for publication Oct. 1, 1956.

†Deceased.

MATERIAL AND METHODS

The methods of subjecting early embryos of Amblystoma (blastula and early gastrula stages) to extremely low oxygen tensions (1 to 0.05 per cent) and to atmospheres with large amounts of CO_2 (17 to 40 per cent) have been previously described.[1, 2] Further publications[3] describe the methods of subjecting Amblystoma embryos (ranging from early cleavage to tail bud stages) to various concentrations of indolebutyric acid.

RESULTS

Developmental defects ranged from complete failure of mouth organs to form (Fig. 2) to gross distortions of the oral cavity with groups of teeth developing in abnormal locations. In some, the oral epithelium was thickened either in the mandibular arch only or in the mandibular and maxillary arches (Fig. 3). The hyoid, Meckel's and palatoquadrate cartilages were greatly distorted and might even have been fused, forming a solid sheet of cartilage unilaterally. One animal, case 4 NE 6-1 (Fig. 4), showed the absence of the hyoid and palatoquadrate cartilages. The Meckel's cartilages were close together. Teeth formed between the cartilages with no apparent relationship to the oral cavity. The trabecular cartilages were small, and few teeth were associated with them. This animal also showed abnormal brain formation. Case 3 CO_2 E3-1 showed a greatly reduced size of Meckel's, hyoid and palatoquadrate cartilages. The mouth was larger than normal, and the epithelium associated with the mandibular portion of the mouth was thicker than usual. This animal also had a median telencephalon with large cavity. Case 2 CO_2 E-1-2 showed an abnormally large mouth. In only one area was there any cartilage with teeth. That cartilage resembled Meckel's cartilage. It was median and appeared to grow up from the floor of the mouth (Figs. 5 and 6). In addition, there was cyclopia, monorhyni, and median telencephalon.

INDOLEBUTYRIC ACID GROUP

The changes in this group were somewhat similar to those described for the low O_2, high CO_2 groups.

In some animals, almost no mouth existed. Case A9-154, in addition to partial fusion of the eyes and suppressing of the normal bilaterality of the forebrain, illustrated the fusion of cartilages of the head and the failure of normal mouth formation. In one area, a small group of teeth had formed in a mass of epithelial tissue (Fig. 7). Most of the cases in this group showed macrostomia (Fig. 8). All of the cartilages of the head were distorted to some degree. Case A-9-127 (Fig. 8) showed failure of the palatoquadrates to form and an elongation of Meckel's cartilages. The palatoquadrates had not formed in another case either, while in still another there was a fusion and/or distortion of many of the head cartilages, so that it was difficult to determine which were present. One had the impression that several accessory cartilages had formed and fused together. Case IB 5-121 showed a failure of the hyoid cartilages to form and a diminution in size of the abnormally placed Meckel's cartilages. Teeth were present, but they were fewer in number than normal and abnormally located.

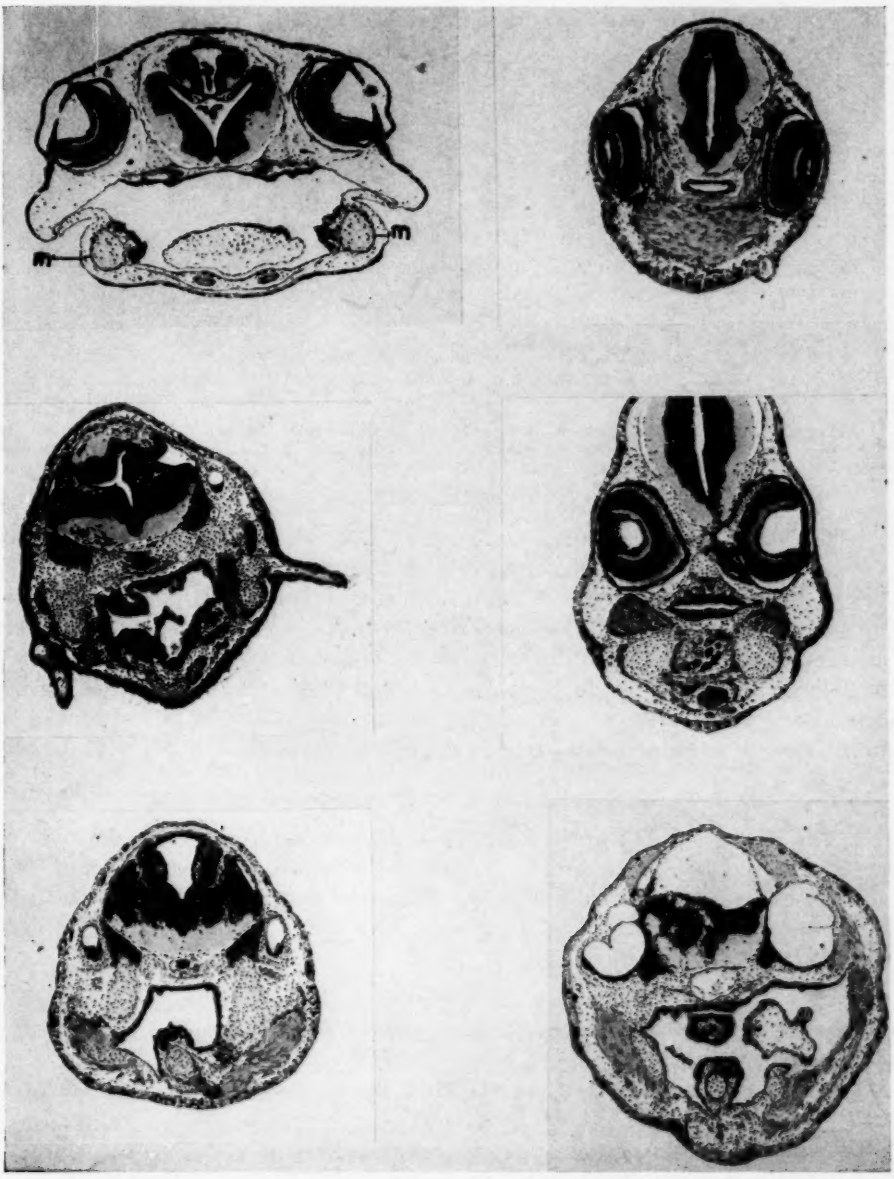

Fig. 1.—Photomicrograph of transverse section of head of normal adult embryo. *M*—Meckel's cartilages.

Fig. 2.—The mouth parts are completely missing in this animal.

Fig. 3.—The cartilages are distorted and fused. The mouth is misshapen and the mucous membranes are thicker than normal.

Fig. 4.—The teeth have formed between Meckel's cartilages without reference to the mouth, which is small and lined by thickened epithelium.

Figs. 5 and 6.—Meckel's cartilage is median and appears to push up from the floor of the mouth.

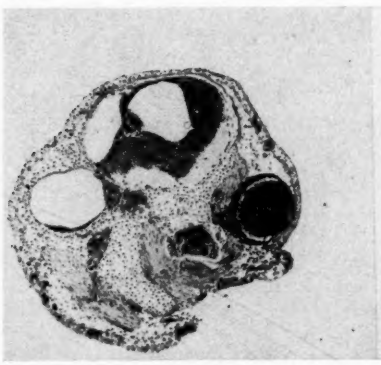

Fig. 7.

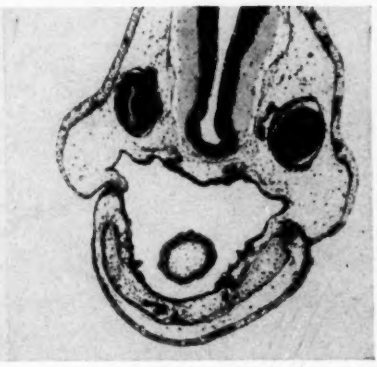

Fig. 8.

Fig. 7.—This section shows a mass of teeth just beneath the eye.
Fig. 8.—This section shows an abnormally large mouth and other anatomic distortions.

DISCUSSION

The experimental results reported here show that with Amblystoma eggs, slowing down of the developmental rate at critical periods produces abnormalities of the jaws and mouth. While previous communications point to the suppression of bilaterality of other head structures (i.e., eyes, forebrain, nasal pits) as being due to a direct effect of CO_2, low O_2, or indolebutyric acid on prechordal mesoderm, we should like to point out here that these oral abnormalities may be due to ectodermal changes. These may be due to a failure of neural crest cells to migrate or to an abnormal rate of migration of the cells of the neural crest.

SUMMARY AND CONCLUSIONS

The effects of CO_2, low O_2, and indolebutyric acid on the development of the mouth and jaws is described. Variations in mouth formation, ranging from astomia to macrostomia, are produced. Abnormal cartilage and tooth formation also results from this type of treatment. No twinning or head duplications occurred.

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THE STRUCTURE OF ROSTRAL TEETH AND THE ROSTRUM OF PRISTIS MICRODON

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THE rostral teeth of the Pristidae exhibit a number of peculiarities which have interested dental anatomists for some time. They are reputed to be composed of vaso-dentin by some[1] and of plici-dentin by others[3]; they are implanted in sockets along the margin of a rostrum, gomphosis being one of the rarer methods of attachment in pisces; they are reputed to be held in these sockets by cementum[4]; and they are one of the few examples as Owen says "of 'permanent teeth' to be met with in the whole class," i.e., they grow from persistent pulps. Owen seems to have been the only person to suggest that they were not teeth at all " . . . and the rostral teeth of Pristis (if these modified dermal spines may be so called). . . ."[1] An examination of these teeth was carried out to investigate the structure of the dentin during an histologic survey of the types of dentin which may occur in the animal kingdom.

MATERIAL AND METHODS

The material consisted of dried museum specimens. In many ways, this was unfortunate, as fixed material would have proved very useful in a study of the development of these rostral teeth. It has not been possible, as yet, to obtain fresh or fixed specimens. Ground sections were produced by routine technics although very thin sections proved difficult to obtain as the tissue tended to "fray" at the edges. The production of decalcified sections was found to be more difficult than experience with other reputedly similar tissues might have led one to expect. It was found to be very difficult to remove all the calcium salts from the tooth when this was treated in bulk. Even with small pieces, the process required at least 10 days in 10 per cent nitric acid, the fluid being changed daily. Normally, decalcification of calcified tissues produces a large initial outflow of calcium into the decalcifying fluid, the amount decreases steadily until an endpoint is reached. In treating the present tissue, however, only a small amount of calcium was removed daily, and this outflow continued constant over a long period. This would indicate that in the museum specimen, the tissue is extremely dense and all but impermeable, an indication which was borne out by the difficulty of obtaining sections of mature tissue once it was embedded in wax. The decalcified sections used were therefore from the less mature portions of the tooth close to the attachment, where the presence of patent canals and canaliculi allowed the free penetration of fluids.

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OBSERVATIONS

The teeth in ground transverse sections present an appearance in certain areas similar to the classical picture of plici-dentin[3] (Fig. 1), or trabecular dentin[2]. Each tooth appears to be composed of a series of systems running longitudinally, each system consisting of a central canal running parallel to the long axis of the tooth, from which originate a series of canaliculi which radiate into the surrounding hyaline matrix. These systems of the central and immature areas of the tooth differ from the classical description of plici-dentin in that: (1) the canaliculi are finer and branch more frequently, (2) there are few if any anastomoses between the canaliculi of the adjacent systems, and (3) the matrix shows no evidence of laminations caused by incremental deposition. The systems toward the tip, and the periphery of the tooth varied from the above description in that the canaliculi if present were not patent, and the central canals had been occluded by a plug of some hyaline material. This would suggest that the systems shown in Fig. 1 are probably immature. The matrix of the peripheral systems is marked by the presence of many dark areas, the positions of which are important as they seem to outline the basic structure of the tissue (Fig. 2). Closer inspection reveals that these dark areas outline a number of round or polygonal bodies, in much the same way as the interglobular areas of dentin outline the globules of mature dentin matrix. A further examination of the whole tooth reveals that the structure seen in the peripheral systems is duplicated in the central systems, although here the polygonal bodies are separated from each other either by a tissue of almost equal refractive index, or by canaliculi. Furthermore, the structure is also present in the tissue in between the systems.

The question arises as to whether these bodies, which appear round or polygonal in transverse sections of the tooth, are spheres or transverse sections of rods. Longitudinal ground sections, due in all probability to their thickness, and the consequent superimposition of one structure upon another, showed little or nothing, so resort was made to decalcified sections.

Decalcified transverse sections of the immature tissue confirmed the observations that the matrix was composed of round or polygonal bodies embedded in some cementing substance. There was, however, less evidence of this later as each body was bordered for a portion of its periphery by a clear unstained area. This apparent space separated the body from the cementing substance not from the adjacent bodies (Fig. 3). Longitudinal sections showed that the tissue is composed of rods which appear to be continuous from their point of formation in the germinal tissue to the external surface of the tooth (Fig. 4). It was not possible to distinguish any inter-rod tissues in these sections but this a common feature of longitudinal sections, viz., human enamel. There is no doubt that both rod and inter-rod tissues do exist as witnessed by the apparent space separating them, and also by the fact that where the sections are very thin, the rod contents separate from the section leaving on the slide only the reticulum of inter-rod tissue.

Close to the central canal of each system, the matrix tends to lose its rod structure and to become homogeneous, and it is in this area that the canaliculi are best seen. These canaliculi appear to be in continuity with some of the apparent spaces between the rod and inter-rod tissue, thus accounting for the very rich canalicular branchings which have been noted in ground sections.

The rods, which are approximately 5 microns average in diameter, are themselves composed of small globules or granules which stain deeply with hematoxylin. They give the appearance on an enlarged scale of the ultra-lattice which has been noted within the human enamel prism.[7] In contradistinction, the inter-rod tissue seems to be homogeneous.

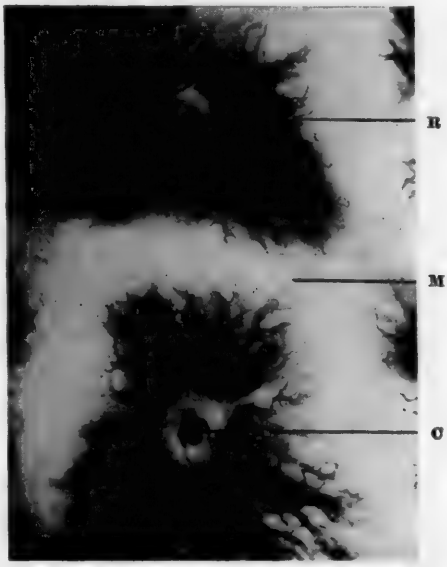

Fig. 1.—Ground transverse section of rostral tooth showing 2 central systems cut transversely, unstained. *C*—central canal of a system; *R*—radiating canaliculi; *M*—the intersystem matrix. (Orig. mag. ×178.)

The rostral teeth are developed upon a highly convoluted, persistent germinal tissue which, it is presumed, forms not only the bed upon which the tooth develops but also the contents of the canals which penetrate the tooth and form the central canals of the systems. The germinal tissue presumably increases in size gradually and so forms a conical tooth, the base of which becomes progressively larger, commensurate with the increase in size of the fish. An interesting feature of this increase in girth of the base of the tooth is that it seems to result in part also from the apposition of tissue onto the external surface. This is evidenced by the observation that the external surface of the "root" of the tooth is covered by a layer of tissue similar to that which lines the central canals, presumably immature tissue; canaliculi open onto this surface, and rods originate from it. Thus, though the main directions of growth of this tooth are from the base toward the tip, and from the periphery of each

Fig. 2.

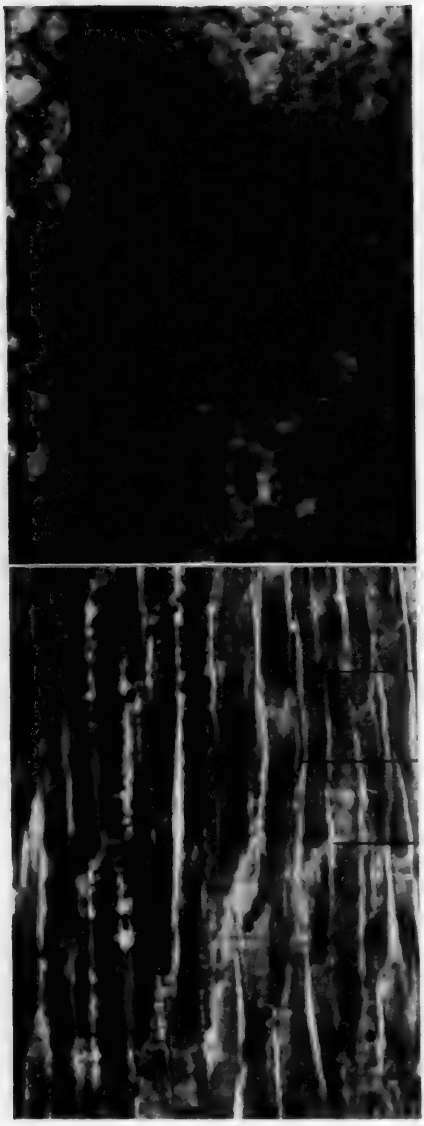

Fig. 3.

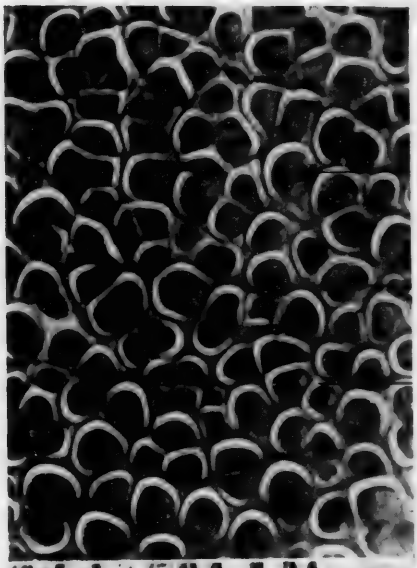

Fig. 4.

Fig. 5.

Fig. 2.—Ground transverse section of a rostral tooth, unstained, to show the structure of the matrix of the systems at the periphery of the tooth. *B*—Round or polygonal translucent bodies distinguishable from *I*—the opaque intervening tissue. (Orig. mag. ×1,500.)

Fig. 3.—Decalcified transverse section of a rostral tooth, stained Masson's trichrome. Note crescentic unstained area which separates the granular rod tissue from the more homogeneous inter-rod matrix, for only a portion of its circumference. There is a striking similarity between the disposition of the tissues here and those in human enamel. *R*—granular rod tissue; *I*—inter-rod tissue; *S*—crescentic unstained area. (Orig. mag. ×1,500.)

Fig. 4.—Decalcified longitudinal section of a rostral tooth, stained Masson's trichrome. This shows that the tissue is indeed composed of rods each bounded by an unstained area. *R*—rod tissue; *S*—unstained areas; *I*—inter-rod tissue. (Orig. mag. ×1,000.)

Fig. 5.—Decalcified longitudinal section of a rostral tooth showing the area of attachment, stained Masson's trichrome. *M*—the hyaline matrix of the tooth; *C*—canaliculis originating on the tooth periphery; *E*—the remains of the stratified squamous epithelium of the attachment. (Orig. mag. ×1,100.)

system toward each central canal, the tissue on the periphery of the tooth develops from within outward. Yet the tissue is similar in structure throughout (Fig. 5).

The presence of a formative layer of tissue surrounding the root of the tooth may explain the assertion that these socketted teeth are held in place by cementum,[4] but there was no cementum present on the specimens examined. The socket appeared to be lined by the remains of stratified squamous epithelium apparently continuous with the germinal tissue responsible for the apposition of tooth substance to the external surface of the root.

Though on a slightly larger scale, it was found that the rostrum, on which these teeth are set, was built up on exactly the same plan as the individual tooth. Large canals run the whole length of the rostrum, each canal being surrounded by matrix in which radiate canaliculi, the matrix being composed of these peculiar rods and inter-rod tissue.

DISCUSSION

The tissue of the rostral spines of *Pristis microdon* does not seem to simulate any other piscine tissue so far described in the literature. It can be clearly differentiated from cementum, bone, cartilage, and all types of dentin so far described. The only tissue to which it bears a resemblance is mammalian enamel, although even here the resemblance is slight.

The finding of this apparently isolated example of a peculiar tissue leads to some questioning as to its origin, and as to whether other similar tissues, as yet unidentified, actually exist in other animals. The literature seems to contain but a single reference to the presence of a tissue of prismatic structure in a fish, so that one can but conjecture on the distribution of this type of tissue. Peyer[6] investigated the histologic structure of the tooth plates of certain ceratodians, and found that in the young tooth plate the outermost layer of tissue, about 8 microns thick, showed a prismatic structure. Lison[5] refuted the suggestions that there is a separate outer layer even in the tooth plates of the very young, and that any portion of the tissue has a prismatic structure. As he was working with fixed developing tissue, it is unlikely that he would have missed a surface layer; on the other hand he figures only longitudinal sections of the tissue and, as pointed out earlier, a prismatic structure is more difficult to recognize in these sections than in transverse sections as figured by Peyer.

At the moment, therefore, it can be assumed that these rostral spines present the first definite evidence of the presence of a prismatic calcified tissue in pisces. So far, attempts to establish whether the matrix of the tissue is of a keratinous or collagenous nature have been inconclusive.

CONCLUSIONS AND SUMMARY

A study of the rostra and spines of *Pristis microdon* suggests that the structure of these organs in the Pristidae is quite different from the classical

concept. This concept was that the spines were composed of a type of dentin which showed resemblances to similar dentin found in other fish and also to the other types of dentin.

The rostra and spines are, in fact, almost identical in structure, being composed of innumerable polygonal prisms or systems which run parallel to long axis of the organ, each system being cemented in close apposition to its neighbors. Each system is seen in transverse section to be composed of a more or less hyaline matrix surrounding a central canal. From this canal, arise small lateral branches which penetrate into the surrounding matrix where they ramify into very fine terminal branches. Thus far, the description is similar to certain so-called forms of dentin. The matrix of this tissue, however, differs markedly from a dentin matrix in that it consists of rods and an interrod cementing substance. The rods run parallel to the central canal and are bounded for a portion of their periphery by a rod sheath similar in distribution to the prism sheath seen in human enamel. No conclusions can be drawn at present as to the true nature of this tissue nor of its possible evolutionary relationships.

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INVESTIGATIONS OF THE INFLUENCE OF VASCULARITY AND INNERVATION ON TOOTH RESORPTION AND ERUPTION

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ALTHOUGH the phenomena of tooth eruption and resorption have received considerable attention in clinical and experimental research,[1-27] the many theories that have been advanced to explain these processes attest to the existing confusion and need for additional study.

Based on findings presented in the more comprehensive investigations, it would seem that vascular influences are of major importance.[15, 28] Consequently, the present study was designed primarily to evaluate the picture of resorption and eruption following section of the common carotid artery and cervical sympathetic chain.

EXPERIMENTAL PROCEDURE

The diphyodont nature of the rabbit dentition makes this animal an ideal subject for radiographic and histologic studies of deciduous tooth resorption and exfoliation. Unfortunately, however, such studies on the posterior teeth cannot feasibly be extended to include observations on eruption, due to the difficulty of making direct measurements.

In order to initiate an alteration of blood supply to the mandible, 2 groups of 12 rabbits each were subjected, respectively, to unilateral ligation of the common carotid artery and unilateral resection of the superior cervical sympathetic ganglion. A third procedure to eliminate afferent innervation was accomplished on 12 additional rabbits by sectioning the inferior alveolar nerve. In all instances, the animals were of New Zealand strain, equally divided by sex, and operated upon at 7 days of age. Anesthesia was with intravenous sodium pentobarbital (4 mg. per 100 Gm. of body weight). Following sacrifice at periods ranging from 3 to 23 days postoperatively, the mandibles were split at the symphysis and the experimental and control sides prepared for histologic examination.

Standardized x-ray technics were employed, extreme care being taken to direct the rays perpendicular to the center of the mandible. In making

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caliper measurements of posterior tooth growth from these radiographs, the distance from the tip of the highest cusp to the inferior border of the mandibular canal provided standard points of reference.

The difficulty of recording eruption rates in the posterior teeth of rabbits, suggested the advisability of utilizing the incisor teeth of rats. A group of 36 nonoperated Wistar albino animals, age 70 days, were divided equally by sex and observed for a period of 60 days. All animals were maintained on a normal, adequate diet. The maxillary and mandibular incisors of each animal were initially marked at the gingival crest of the labial surface by means of a small revolving diamond instrument. Radiographs were then taken at 7-day intervals, light ether anesthesia being employed in order to obviate any chance of movement during exposure.

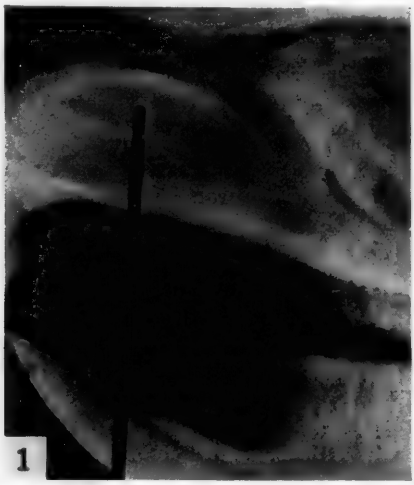

Fig. 1.—Roentgenogram showing planes of reference for calculation of incisor eruption rates in the rat.

A survey of the literature on measurements of incisor eruption rates in the rat reveals that previous in vivo studies employed a technic of direct gross observation.[10, 11, 14, 20] Obviously, the data presented in such investigations may be open to question because of the physiologic variation in gingival crest position and the cusp tip from which measurements were routinely taken. It seemed necessary, therefore, to devise a method by which all measurements of eruption could be made from a more stable point of reference, namely, the alveolar bone crest (Fig. 1). As shown, a 90-degree bisection of the occlusal plane by means of a line drawn from the anterior point of the alveolar crest provided a relative standardized means of reference. Eruption rates were then determined by measuring the distance from this point of reference to the incisor marking. The observed eruption rates are recorded in Table I.

Following the establishment of base line measurements under control conditions, the rats were divided into 3 groups and subjected, respectively, to

TABLE I
PREOPERATIVE AND POSTOPERATIVE ERUPTION RATES OF WISTAR ALBINO RATS

GROUP	NUMBER OF RATS	WEEKLY ERUPTION RATES IN MM.	
		MAXILLARY INCISOR	MANDIBULAR INCISOR
Control	36	2.31	3.60
Common carotid	12		3.60
Inferior alveolar nerve	12		4.2
Cervical sympathectomy	12		3.5

common carotid artery ligation, cervical sympathectomy, and section of the inferior alveolar nerve. Following this procedure, routine measurements of eruption were continued for an additional period of 8 weeks. Due to the anastomosis of the blood supply and bilaterality of innervation in the anterior midline area,[29-33] the operative procedures were done bilaterally.

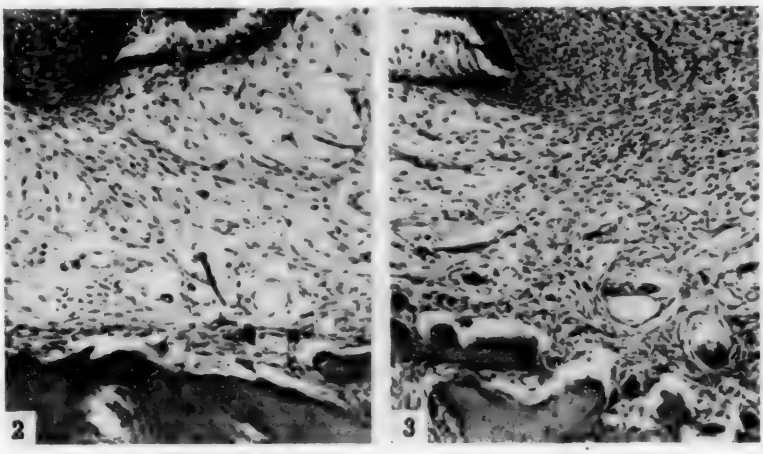

Fig. 2.—Operated side of the mandible following unilateral section of the common carotid artery. The reduced osteoclastic activity at the alveolar crest inferior to the fundus of the first molar is clearly evidenced.

Fig. 3.—Nonoperated side of the mandible showing normal osteoclastic activity.

FINDINGS

In the group of rabbits subjected to right common carotid artery ligation, there was noted on the operated side a decrease in osteoclastic activity as well as a reduction in periodontal and pulpal vascularity (Figs. 2 and 3). The latter finding is apparently transient inasmuch as it was evidenced only in the earlier postoperative periods. These teeth showed no differences either in their time of shedding or in their axial dimension when compared with the nonoperated control side. It is of additional significance to note that the incisor eruption rate of rats following bilateral ligation of the common carotid artery was also comparable to the control group (Table I).

In that group of rabbits subjected to unilateral sectioning of the inferior alveolar nerve, no essential histologic differences in the dental and supporting

tissues were noted between the operated and nonoperated sides of the mandible. In striking contrast to these findings, the mandibular incisor eruption rate in rats was markedly increased following bilateral section of the inferior alveolar nerve (Table I).

Rabbits subjected to unilateral cervical sympathectomy showed an increased osteoclastic activity in the region of the resorbing deciduous and erupting permanent teeth (Figs. 4 and 5). A corresponding increase in pulpal and periodontal vascularity was also noted (Figs. 6 and 7). Deciduous tooth exfoliation and axial dimensions of permanent posterior teeth in both experimental and control animals were essentially the same. In the rat group subjected to bilateral sympathectomy, the eruption rate of the mandibular incisors was unaltered (Table I).

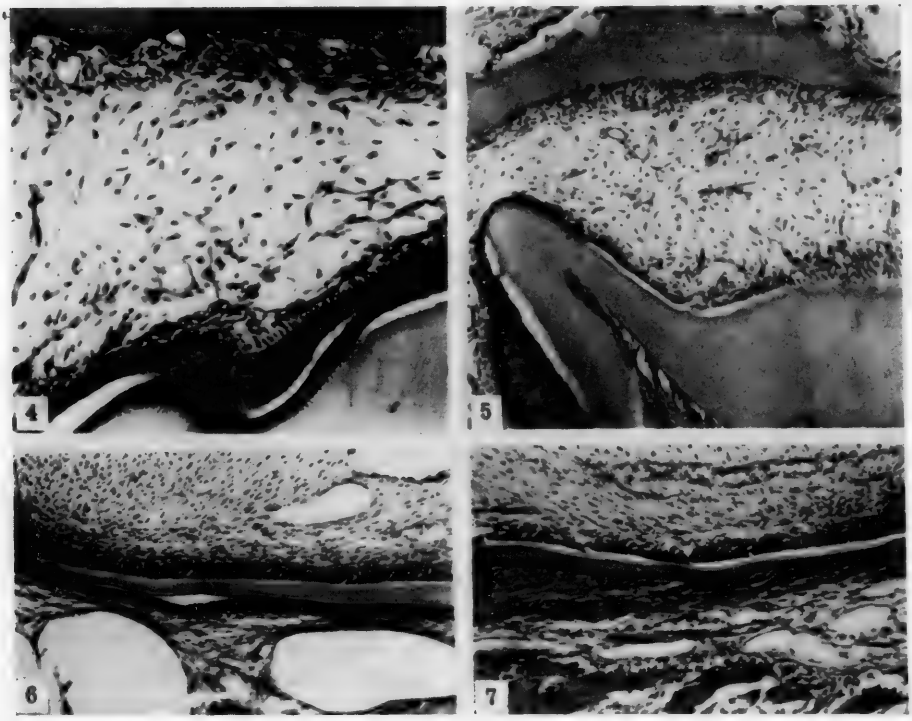

Fig. 4.—Section of mandible from operated side following unilateral cervical sympathectomy in rabbit. Note the increased osteoclastic activity and vascularity in the area of the resorbing deciduous tooth.

Fig. 5.—Section of mandible of nonoperated side showing normal osteoclastic activity and vascularity.

Fig. 6.—Operated side of the mandible following unilateral cervical sympathectomy. Note the increased vascularity of pulpal and periodontal tissues.

Fig. 7.—Section of the mandible of the nonoperated side showing normal degree of vascularity of periodontal and pulpal tissues.

Certain incidental findings were noted which, because of their variance with the so-called "pressure theory" of tooth resorption, are worthy of mention. Briefly described, these included a highly regular surface of resorption

and an unaltered connective tissue architecture between and adjacent to the permanent tooth cusp and the deciduous root (Fig. 8). Another interesting finding was that the unerupted permanent teeth showed no encapsulation by alveolar bone. This may be of some significance inasmuch as earlier investigators suggested that the alveolar crypt either completely surrounded the developing permanent tooth or was partially resorbed only in the area of the adjacent deciduous tooth.[3, 4]

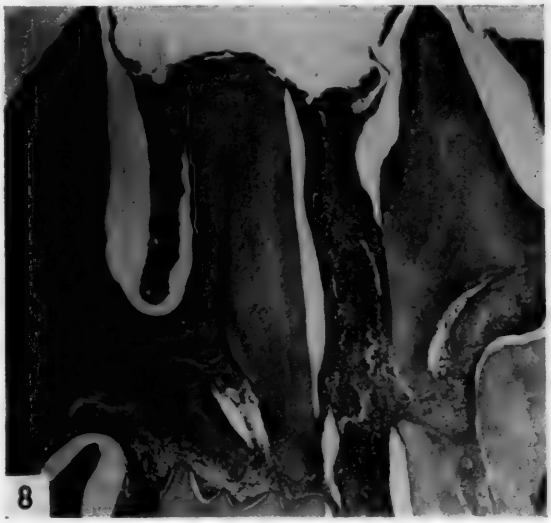

Fig. 8.—Resorbing deciduous and developing permanent teeth showing absence of aveolar bone crypts in unerupted dentition.

DISCUSSION

No evidence was noted to support the contention of several previous investigators[1, 4] that pressure is an essential factor in the resorption and exfoliation of the deciduous dentition. The logic of the "pressure theory" was questioned as early as 1838 by Hunter.[3]

Comprehensive reports in the literature[1, 3, 12, 28] are seemingly agreed that vascularity, and perhaps innervation, may be major factors in tooth resorption with consequent exfoliation. Lending support to the "vascular" hypothesis is the finding in the present study of a direct relationship between osteoclastic activity and the degree of vascularity in animals subjected to such procedures as cervical sympathectomy and common carotid artery ligation.

The transient histologic changes noted in animals subjected to ligation of the right common carotid artery was of particular interest. It will be recalled that marked reduction of osteoclastic activity, as well as reduced vascularity of the pulpal and periodontal tissues, were apparent only during the early postoperative periods, prior to the eighth postoperative day. Such transient histologic changes may be explained, in part, by the generally accepted phenomenon of retrograde blood flow.[34] As suggested by Batson, the vertebral

arteries serving as collaterals to the internal carotid branches of the common carotid artery have the potential of establishing considerable pressure and blood volume in the circle of Willis. Consequently, a relatively normal supply of blood to the tissues of the mandible is established.

The negative findings in those rabbits, subjected to unilateral section of the inferior alveolar nerve, are at variance with reports of Leist[13] and Edwards and Kitchin[14] who described increased vascularity following the same operative procedure. Lending support to observations in the current investigation is the report of Taylor[27] which provided strong evidence that autonomic fibers are not associated with the inferior alveolar nerve. It is quite reasonable to expect, therefore, that no alterations in vascularity would follow sectioning of this structure.

The unaltered pattern of incisor eruption in rats following bilateral ligation of the common carotid artery may be explained by the same process of retrograde flow that follows unilateral artery ligation. Although bilateral cervical sympathectomy is likewise not a factor in causing any change in incisor eruption rate, it is apparent that the phenomenon of retrograde blood flow is not pertinent to this experimental series. However, since no histologic study was made in this group, it is not possible to state whether sympathectomy in the rat causes an increased vascularity comparable to that described in the rabbit.

The accelerated eruption of rat incisors as observed in the current study following section of the inferior alveolar nerve was also noted by previous investigators.[1-15] In explanation of this finding, Leist[13] suggested some type of neural mechanism directly associated with tooth eruption. Wetzel,[10] and Moral and Hosemann,[12] on the other hand, suggested that the influence of the inferior alveolar nerve on eruption may be due indirectly to sensory impulses conducted by reflex action from periodontal tissue. As explained by Taylor and Butcher,[15] removal of this sensory innervation would render the incisor tooth more susceptible to accidental fracture and chipping with a consequent shortening and increased rate of eruption.

Demonstrating that unopposed rat incisors do show a markedly accelerated rate of eruption, Schour and Medak[24] excised a portion of the clinical crown of the maxillary incisors and reported a significant increase. In a somewhat different manner, other investigators have likewise shown that the rapidity of attritional wear of rodent incisors is directly related to their rate of eruption.[10, 11]

Although it was shown in the current study that rats subjected to inferior alveolar nerve section have an acceleration of incisor eruption, the observation of frequent tooth chipping is evidence that an interruption of sensory innervation to the periodontal tissues is perhaps the actual influencing factor.

SUMMARY AND CONCLUSIONS

Resorption and eruption of the dentitions as influenced by sectioning of the common carotid artery and the inferior alveolar nerve as well as resection

of the cervical sympathetic chain were studied in a series of 36 rabbits and 36 rats.

On the basis of histologic and radiographic findings, the following conclusions are suggested:

1. There is possibly a direct relationship between osteoclastic activity and the degree of vascularity in dental and supporting tissue.

2. Resorption in deciduous teeth of rabbits is seemingly sufficiently advanced by the seventh day of life so that any alteration of osteoclastic activity has little or no effect on exfoliation.

3. Eruption rates of rat incisors do not appear to be sensitive to even fairly considerable alteration of blood flow to adjacent tissue.

4. Bilateral sectioning of the inferior alveolar nerve in rats is followed by an acceleration of mandibular incisor eruption.

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THE INFLUENCE OF SYSTEMICALLY ADMINISTERED TRYPAN BLUE ON PRENATAL DEVELOPMENT OF RATS AND MICE

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DISTURBANCES in the development of teeth have been attributed to a wide variety of disease processes.[1] Although such relationships have been established largely by clinical observation, the laboratory animal provides substantial supporting evidence of the marked susceptibility of developing teeth to many systemic disturbances.[2-3] It is of interest to note, however, that most studies of enamel and dentin hypoplasia are related to the postnatal period.

Relative to the question of prenatal influences on dental development, it is generally accepted that deciduous teeth are rarely affected by hypoplasia. However, the findings of Stein,[5] Sheldon, Bibby, and Bales,[6] and Kreshover, Clough, and Bear[7] suggest that intrauterine disturbances in odontogenesis can occur with rather significant frequency. Consistent with these observations in human beings is the retarded dental development reported by Mellanby in the young of rats maintained on vitamin A deficient diets,[8] and the abnormal amelogenesis that can be seen in the offspring of diabetic and pyrexic rats.[9, 10]

Although placental permeability to various noxious agents, including viruses, may play an essential part in the pathogenesis of congenital abnormalities, the possibility also exists that such defects may be due to metabolic dysfunction incident to the maternal disease. While no definitive information has been forthcoming relative to the mechanism by which malformations are caused in children born of mothers with rubella or other diseases, some attempt at explanation has been made. Hypothesizing that many congenital defect syndromes are causally related to a combination of plasma proteins with abnormal metabolites, Gillman, Gilbert, and Gillman explored the question of whether trypan blue might simulate the particulate matter derived from tissue breakdown.[11] Inoculating pregnant rats with established doses of the dye at prescribed stages of gestation, these authors reported an unusually high incidence of congenital defects varying from absent eyes and tails to spina bifida and hydrocephalus. Unfortunately, little attention was given to the dentition.

The present study was designed in order to determine whether maternal metabolic dysfunction related to trypan blue administration can initiate congenital dental defects.

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TABLE I

TREATMENT	TIME OF INJECTION	TOTAL NO. ANIMALS TESTED	NO. AND (%) OF CONFIRMED PREGNANCIES	NO. AND (%) OF LITTERS CONTAINING ABNORMAL FETUSES	NO. AND (%) OF LITTERS CONTAINING RESORBED OR DEAD FETUSES	TOTAL NO. OF FETUSES*	NO. AND (%) OF MALFORMED FETUSES	NO. AND (%) OF RESORBED OR DEAD FETUSES	NO. AND (%) OF NORMAL FETUSES	AVERAGE NO. OF FETUSES PER MOTHER
					Rats					
Trypan blue	1-49 days prior breeding plus 3-14 days post-breeding	27	19 (70.4%)	3 (15.8%)	4 (21%)	144	10 (6.9%)	16 (11.1%)	118 (81.9%)	7.6
Trypan blue	6-9 days postbreeding	47	38 (80.8%)	22 (57.9%)	17 (44.7%)	324	40 (12.3%)	62 (19.1%)	222 (68.5%)	8.5
Trypan blue	9-21 days postbreeding	77	51 (66.2%)	2 (3.9%)	13 (25.5%)	461	2 (0.4%)	32 (6.9%)	427 (92.6%)	9.0
Control	—	25	23 (92%)	0	3 (13%)	237	0	3 (1.3%)	234 (98.7%)	10.3
					Mice					
Trypan blue	6-8 days postbreeding	112	55 (49.1%)	21 (38.1%)	38 (69.1%)	431	35 (8.1%)	145 (33.6%)	251 (58.2%)	7.8
Trypan blue	9-17 days postbreeding	110	29 (26.4%)	3 (10.3%)	21 (72.4%)	224	6 (2.7%)	72 (32.1%)	146 (65.2%)	7.7
Control	—	68	42 (61.8%)	5 (12%)	21 (50%)	336	9 (2.7%)	51 (15.2%)	276 (82.1%)	8.0

*Including sites of resorption.

MATERIALS AND METHODS

One hundred and twenty-four albino rats were injected subcutaneously with 1.0 ml. of a 1 per cent solution of trypan blue on each of 2 or 3 successive postbreeding days. In accordance with this schedule, the time of initial administration ranged from the sixth to the twentieth day of presumed pregnancy. An additional 27 rats received 1 or more injections of dye both prior to and after breeding.

Similar procedures were carried out on 217 Swiss mice. All but 12 of this series were given single, 0.25 ml. injections of dye at postbreeding intervals of 7 through 17 days. The remaining animals received 0.5 ml. of trypan blue on either the sixth, seventh, or eighth day after copulation.

Twenty-five control rats and 68 control mice were inoculated with sterile distilled water at times corresponding to the schedule in the experimental series.

In all experimental and control groups, the beginning of pregnancy was recorded as that time when spermatozoa were found in vaginal smears of rats, or when a vaginal plug was noted in mice.

Whenever possible, exploratory laparotomies were done on animals shortly before their estimated time of delivery, and fetuses removed for histologic study. Exceptions occurred when rats and mice delivered spontaneously before cesarean section could be accomplished.

FINDINGS

Trypan blue, administered to pregnant rats and mice, was found to have a rather pronounced effect on both the course of pregnancy and fetal development (Table I). In many instances, animals failed to show a progression of pregnancy beyond the appearance of vaginal plugs or spermatozoa in smears. Although dye injections are seemingly related to this finding, it is conjectural whether a mere failure of conception occurred or whether there was actual interruption of established pregnancy. Further difficulty in interpretation is presented by the unusually low incidence of confirmed pregnancy in the control mice. Since previous observations on apparently normal mice showed a 93 per cent incidence of successful pregnancy following vaginal plug formation,[12] it may be considered that the handling required for injections with distilled water caused some disturbance of pregnancy.

With respect to the finding of abnormal, resorbed, or dead young, it is significant that the incidence was appreciably higher in animals receiving trypan blue during the earlier stages of gestation. It should be noted, however, that the number of such abnormalities is much less when considered in terms of individuals affected rather than litters. Of some additional interest is the rather high incidence of abnormalities in the control group of mice. As suggested previously, it is probable that this species is unduly sensitive to handling whereas the pregnant rat is relatively unaffected.

The types of congenital malformations, as well as their incidence, were quite similar in both groups of experimental animals. Although exencephaly

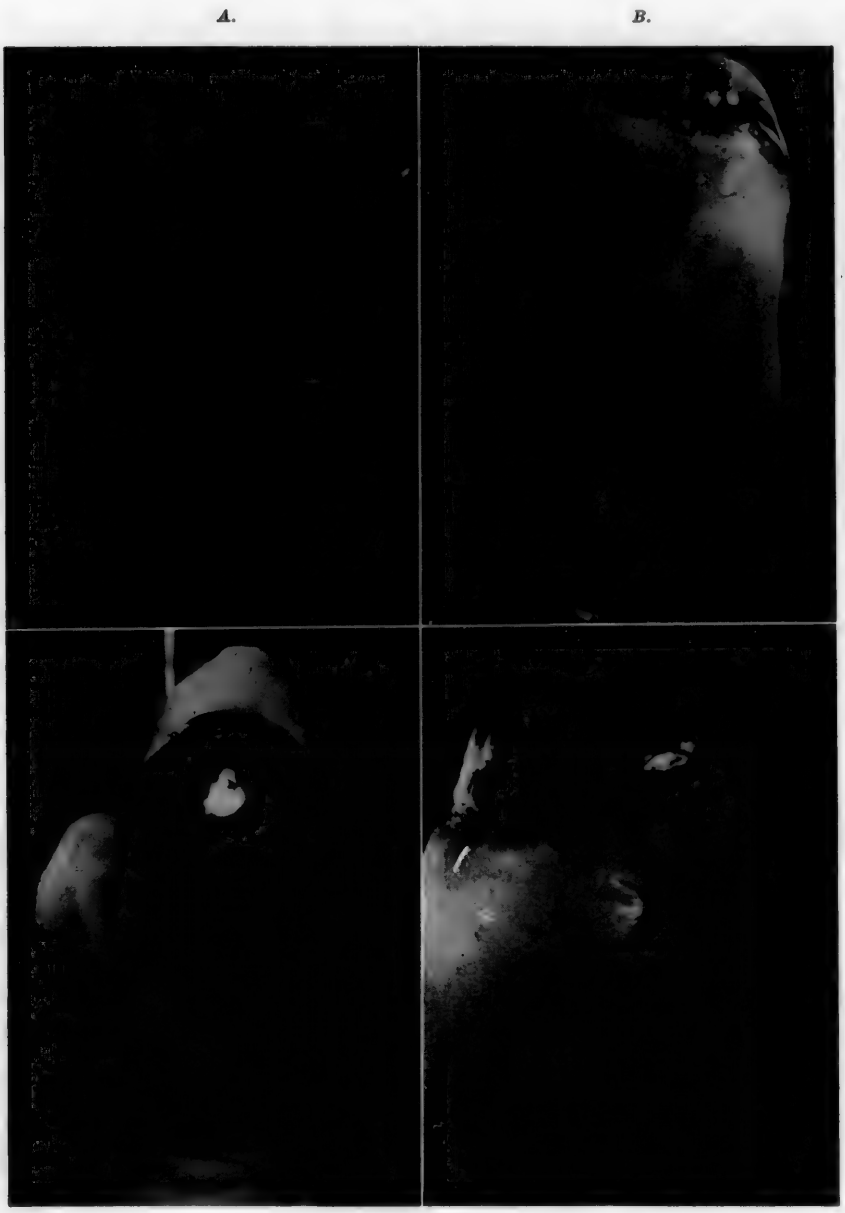
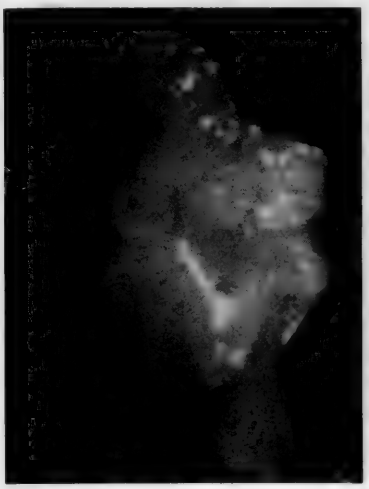


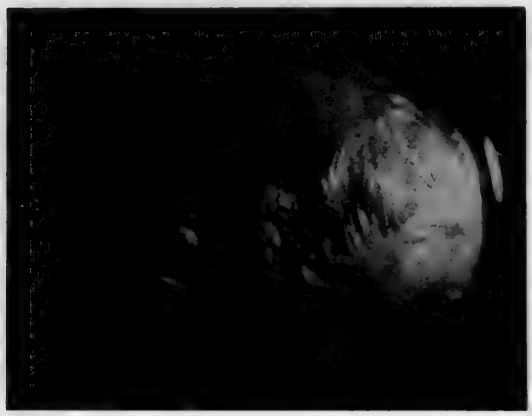
Fig. 1.—*A*, Normal appearing young of a control rat.

B and *C*, Lateral and cephalic views of exencephaly in a 21-day fetus of a rat injected with 1.0 ml. of trypan blue on the seventh, eighth, and ninth days of gestation.

D, Exencephaly in an 18-day fetus of a mouse injected with 0.25 ml. of trypan blue on the seventh day of gestation.



A.



B.

C.

Fig. 2.—*A*, Hydrocephalus in a 17-day fetus of a mouse injected with 0.25 ml. of trypan blue on the seventh day of gestation.

B, Stubby, screw tail in a 21-day fetus of a rat injected with 1.0 ml. of trypan blue on the seventh and eighth days of gestation.

C, Fused hind legs of a 20-day fetus of a rat injected with 1.0 ml. of trypan blue on the eighth and ninth days of gestation.

(Fig. 1) was most frequent in occurrence, there were repeated instances of hydrocephalus, missing or rudimentary tails, anophthalmia, and clubbed feet (Fig. 2). Only rarely was spina bifida observed.

Gross examination of the oral cavity of each experimental and control animal showed no abnormalities. Although extended postnatal observations were made in but a few cases, there was no evidence of altered eruption rates of the teeth. Histologic study of the maxillae and mandibles likewise failed to reveal any disturbances in odontogenesis. The ameloblasts of both the incisor and molar teeth were clearly defined and overlay a normal appearing organic enamel matrix. The dentin, predentin, and pulpal tissues were similarly free of abnormalities.

DISCUSSION

The present study, although demonstrating teratogenic properties of trypan blue, shows a lesser incidence of defects than reported by Gillman, Gilbert, and Gillman.[11] It is of interest to note that the later during pregnancy that the dye was injected, the fewer were the instances of malformed, resorbed, and dead young.

Despite the varied types and severity of malformations noted, there was a consistent absence of dental abnormalities. While this seemed to be evidence of the selective nature of the particular metabolic disturbance produced, it was necessary to consider the fact that the time of dye injection preceded the onset of odontogenesis in the fetus. However, such an hypothesis became untenable when it was shown that the maternal incisors were likewise unaffected. Certainly, if a generalized disturbance were to affect amelogenesis or dentinogenesis, teeth of continuous growth would show changes regardless of the chronologic age of the animal.

On the basis of the reported findings, it may be assumed that trypan blue induces a type of metabolic disturbance that is related to specific types of malformations but, for reasons unanswered, spares the developing dentition.

SUMMARY AND CONCLUSION

The injection of trypan blue into female rats and mice resulted in a variety of congenital malformations including exencephaly, hydrocephalus, clubbed feet, and missing eyes and tails. This provided additional evidence that trypan blue can initiate a form of metabolic disturbance that, in turn, may be related to gross defects of development. Examination of maxillae and mandibles showed that the dentition was not included among the susceptible organ sites and tissues. This would suggest that the mere presence of a severe generalized metabolic disturbance does not necessarily imply an untoward effect on the developing dentition.

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STUDIES OF THE COMPOSITION OF TEETH

VI. The Composition of Syrian Hamster Enamel and Dentin Extracted With KOH-Ethylene Glycol

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IN PREVIOUS studies of the composition of Syrian hamsters' teeth, enamel and dentin of incisor and molar teeth from males and females were separated and purified by standard flotation procedures, after which the organic portion of each purified tissue was removed by heating at 900° C. to a constant weight before analyzing for the principal inorganic constituents in the residues.[1] These studies indicated that the heat-labile portion of the tissues, particularly enamel, was greater than might be expected for rodents' teeth and certainly higher than that of human teeth. Furthermore, calcium:phosphorus ratios of ashed hamster enamel and dentin were lower than those found previously for human enamel and dentin subjected either to KOH-ethylene glycol extraction or to ashing before determining inorganic composition.[2] Since the composition of hamster enamel and dentin, subjected to 900° C. until a constant weight was established, deviated from that reported previously for similar tissue of rats and human beings, it seemed probable that the method of treatment prior to analysis influenced the final results. To investigate such an hypothesis, Syrian hamster enamel and dentin, separated and purified by flotation procedures, were extracted with a solution of KOH-ethylene glycol. The residues were then analyzed for their principal inorganic components for comparison with the analytical data obtained previously for similar tissues subjected to 900° C. before analysis.

EXPERIMENTAL PROCEDURES

The teeth used for analysis were obtained from the same Syrian hamster strain as those used previously for analysis of tooth tissues subjected to 900° C.[1] A group of animals, composed of 106 males and 105 females, yielded the quantities of teeth shown in Table I. The teeth were divided into groups as incisors and molars from males and females and enamel and dentin from each group was separated and purified as described previously.[1]

Table I
Source of the Hamster Teeth Used for Analysis

Source of teeth	Animals per group	Mean weight and standard error (gm.)	Range of weight (gm.)	Dry weight of tooth material (gm.)	
				Incisor	Molar
♂	106	93.9 ± 1.05	80 - 128	11.3266	4.9720
♀	105	95.1 ± 1.03	80 - 128	9.6114	5.6807

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The nitrogen content of each type of purified and dried enamel and dentin was determined by standard micro-Kjeldahl analysis.[3] The organic content of each sample was then established as the weight lost during extraction with 5 per cent KOH in reagent grade ethylene glycol[4] in the apparatus illustrated in Fig. 1. Extraction in this apparatus protected against loss of tooth tissues from bumping during boiling or by other accidental causes, assured careful control of temperature, and facilitated washing and collection of the residual inorganic material by centrifugation after extraction was completed. Extraction with

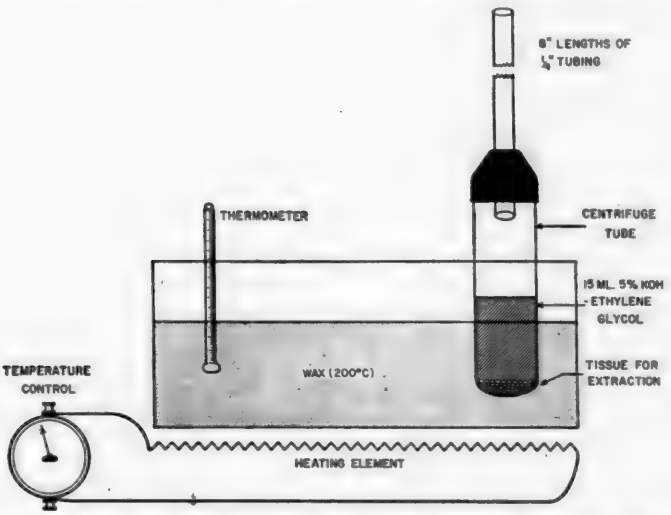

Fig. 1.—An apparatus for extracting the organic matrix from enamel and dentin.

KOH-ethylene glycol for 2 hours at 200° C. was sufficient to remove the organic components from the powdered enamel or dentin. The criterion used for determining complete removal of the organic components was the absence of detectable nitrogen in the inorganic residues. The extracting fluids were tested for the presence of calcium, phosphorus, and magnesium in order to determine whether or not these elements had been removed from the inorganic phase of enamel and dentin during the extraction process. After extraction was completed, the enamel and dentin residues were divided into suitable samples, each of which was dried to a constant weight and dissolved in 0.1 N HCl for analysis of calcium, phosphorus, and magnesium. Calcium was determined by the method of Sobel and Slersky[5] and Sobel and Sobel,[6, 7] phosphorus was determined by the method of Fiske and Subbarow,[8] with modification by Sobel,[9] while magnesium was determined by the method of Orange and Rhein.[10]

RESULTS AND DISCUSSION

The composition of KOH-ethylene glycol extracted hamster enamel and dentin from incisors and molars of males and females is shown in Table II. The nitrogen content of enamel ranged from 0.29 per cent to 0.47 per cent, being higher in the tissue from females than from males. The protein content of the

TABLE II
THE PERCENTAGE CHEMICAL COMPOSITION OF ENAMEL AND DENTIN FROM MALE AND FEMALE SYRIAN HAMSTERS*

SOURCE OF TISSUE	MEAN NITROGEN CONTENT	MEAN PROTEIN CONTENT (% N × 6.25)	MEAN ORGANIC CONTENT BY EXTRACTION	MEAN INORGANIC CONTENT BY EXTRACTION	COMPOSITION OF EXTRACTED TISSUE			Ca:P RATIO
					CALCIUM	PHOSPHORUS	MAGNESIUM	
♂ Incisor enamel	0.35	2.18	5.44	94.56	36.29 ± .33	17.08 ± .53	0.14 ± .01	2.12
♀ Incisor enamel	0.42	2.63	3.47	96.53	35.35 ± .92	17.58 ± .21	0.15 ± .01	2.01
♂ Molar enamel	0.29	1.81	3.05	96.95	35.01 ± .91	16.53 ± .20	0.37 ± .03	2.12
♀ Molar enamel	0.47	2.93	4.62	95.38	35.98 ± .42	17.05 ± .59	0.35 ± .02	2.11
♂ Incisor dentin	3.64	22.75	23.32	76.68	29.89 ± .72	15.64 ± .27	1.46 ± .17	1.91
♀ Incisor dentin	3.70	23.12	23.13	76.87	32.69 ± .10	16.87 ± .06	1.89 ± .21	1.94
♂ Molar dentin	3.80	23.75	22.14	77.86	36.32 ± .35	16.87 ± .26	0.47 ± .01	2.15
♀ Molar dentin	3.76	23.50	20.73	79.27	32.09 ± .83	15.16 ± .42	0.36 ± .003	2.12

*Organic matrix removed by KOH-ethylene glycol extraction.

tissue (calculated as percentage nitrogen times 6.25) ranged from 1.81 per cent to 2.93 per cent. The nitrogen content of dentin did not differ consistently according to source, however, varying only slightly between 3.64 per cent and 3.80 per cent, regardless of whether the tissues were from males or females. The protein content of dentin, calculated as above, ranged only from 22.75 per cent to 23.75 per cent.

The KOH-ethylene glycol extractable portion of enamel did not correlate very closely with the calculated protein content (based on the determined nitrogen content), but the KOH-ethylene glycol fraction of dentin corresponded more closely to the calculated protein content. There was consistently less protein than KOH-ethylene glycol extractable material in enamel. The extractable material in enamel ranged from 3.05 per cent to 5.44 per cent; the extractable components of dentin ranged from 20.73 per cent to 23.32 per cent. The inorganic residue of enamel ranged from 94.56 per cent to 96.95 per cent, while that of dentin was from 76.68 per cent to 79.27 per cent.

A comparison of the data in Table III indicates that the nitrogen and, hence, protein content of hamster enamel is consistently less than the KOH-ethylene glycol extractable portion, even though the latter is mostly organic in nature. However, neither the protein fraction nor the KOH-ethylene glycol extractable fraction of enamel is as great as the fraction lost when the tissue is heated at 900° C. Thus, the protein fraction ranged from 1.81 per cent to 2.93 per cent, the heat-labile fraction ranged from 7.55 per cent to 14.78 per cent, and the ethylene glycol-KOH extractable portion ranged from 3.05 per cent to 5.44 per cent. Therefore, hamster enamel contained approximately 2 per cent more extractable material than protein.

TABLE III
COMPARISON OF THE PROTEIN, KOH-ETHYLENE GLYCOL EXTRACTABLE, AND HEAT-LABILE FRACTIONS OF HAMSTER ENAMEL AND DENTIN

	PROTEIN		EXTRACTABLE		HEAT LABILE*	
Incisor enamel	2.40	2.38	4.45	4.14	12.98	11.34
Molar enamel	2.37		3.84		9.70	
Incisor dentin	22.94	23.28	23.22	22.33	26.65	26.17
Molar dentin	23.62		21.44		25.69	
	Extractable enamel 1.76% more than protein enamel.					
	Heat-labile enamel 8.96% more than protein enamel.					
	Heat-labile enamel 7.20% more than extractable enamel.					
	Extractable dentin 0.95% less than protein dentin.					
	Heat-labile dentin 2.89% more than protein dentin.					
	Heat-labile dentin 3.84% more than extractable dentin.					

*Data condensed from Lobene and Burnett, J. D. Res. 33: 387-496.

The KOH-ethylene glycol extractable fractions of enamel and dentin are not entirely organic, however, for some calcium, phosphorus and magnesium are found in them, even though in small quantities, with phosphorus being most abundant (Table IV). In some instances the inorganic elements found in extraction fluids amounted to as much as 2 per cent of the whole tissue. It must be concluded, however, that the material lost from tooth tissues by heating at 900° C. until a constant weight is reached, exceeded, in all instances, the combined

protein or KOH-ethylene glycol fractions combined with the inorganic components of the extraction fluid. The nature of the heat-labile component is unknown.

TABLE IV
PRINCIPAL INORGANIC CONSTITUENTS OF KOH-ETHYLENE GLYCOL SUPERNATANTS AFTER EXTRACTING ENAMEL AND DENTIN*

TYPE OF TISSUE	CALCIUM		PHOSPHORUS		MAGNESIUM	
♂ Incisor enamel	0.11	0.22	0.37	0.91	0.04	0.02
♀ Incisor enamel	0.45		0.96		0.02	
♂ Molar enamel	0.09		1.78		0.02	
♀ Molar enamel	0.25		0.52		0.02	
♂ Incisor dentin	0.16	0.14	1.37	1.21	0.04	0.03
♀ Incisor dentin	0.10		1.61		0.02	
♂ Molar dentin	0.14		1.15		0.02	
♀ Molar dentin	0.17		0.71		0.04	

*Expressed as a percentage of the whole tissue, including organic matrix.

Little variation was found between protein, KOH-ethylene glycol extractable, and heat-labile fractions of hamster dentin, regardless of its source. In fact, the KOH-ethylene glycol extractable fraction of dentin was slightly less, in some instances, than the calculated protein fraction, and the combustible fraction was not much greater than either the KOH-ethylene glycol extractable or protein fractions. This would seem to indicate that the KOH-ethylene glycol extractable fraction of dentin was either composed largely of a protein whose nitrogen content was less than the theoretical value of 6.25 times the nitrogen content or that dentin responded differently than enamel to heating at 900° C.

The percentage composition of the principal inorganic constituents of extracted tissues is also shown in Table II. The calcium content of the enamel and dentin varied slightly but not consistently according to the source, ranging from 29.89 per cent to 36.32 per cent; phosphorus also varied only slightly, ranging from 15.16 per cent to 17.08 per cent. The magnesium content of the tooth tissues was more variable, ranging from 0.14 per cent in male incisor enamel to 1.89 per cent in female incisor dentin. The calcium:phosphorus ratios of the various tissues is also shown in Table II. Except for incisor dentin, whose calcium:phosphorus ratio was only slightly less than 2.00, the ratios for all the other types of enamel and dentin were more than 2.00.

The calcium and phosphorus content of ashed and KOH-ethylene glycol extracted tissues are compared in Table V. The principal difference to be noted is the low calcium:phosphorus ratios of ashed tissues when compared to those of KOH-ethylene glycol extracted tissues. The differences in the ratios seem to be related to the relatively higher calcium and lower phosphorus content of the extracted tissues as compared to the ashed tissues. Since the ashed tissues contain phosphorus from both inorganic and organic components, it might be expected that they would contain more phosphorus than the extracted tissues. Why the ashed tissues should contain relatively less calcium than the extracted tissue is unknown. Some calcium and phosphorus were found in the extraction

fluid but even when the final composition of the extracted fluid was corrected by the amount of these elements in such fluids, as in Table V, the calcium: phosphorus ratios of the ashed and extracted tissues still differ.

TABLE V
A COMPARISON OF THE CALCIUM AND PHOSPHORUS CONTENT OF ASHED AND KOH-ETHYLENE GLYCOL EXTRACTED ENAMEL AND DENTIN

TYPE OF TISSUE	% CA	% P	Ca:P RATIO
Ashed enamel	33.08	20.19	1.63
Ashed dentin	29.29	20.01	1.46
Extracted enamel	35.65	17.06	2.08
Extracted dentin	32.74	16.13	2.02
Corrected* extracted enamel	35.89	18.00	1.99
Corrected* extracted dentin	32.93	17.68	1.86

*Corrected by the addition of the calcium or phosphorus in the extraction fluid to the calcium or phosphorus in the extracted tissue residue.

SUMMARY

The nitrogen content, KOH-ethylene glycol extractable portions, and the principal inorganic elements in extracted tissue residues have been established for Syrian hamster enamel and dentin from incisors and molars of males and females. The data obtained for such tissues is compared with data obtained previously when subjecting enamel and dentin to 900° C. The protein content of the tissues, as established by nitrogen analysis, was usually less than the material removed by KOH-ethylene glycol extraction. Some calcium, phosphorus, and magnesium, particularly phosphorus, were removed during the extraction process together with the organic components. Neither the protein nor extractable fraction of the tissues was usually as great as that portion of the tissues removed at 900° C. The KOH-ethylene glycol extracted tissue residues contained more calcium and less phosphorus than similar tissue residues heated to a constant weight at 900° C. The calcium:phosphorus ratios of the ethylene glycol extracted tissues were generally higher than those of the heated tissues.

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CLINICAL AND BACTERIOLOGIC STUDIES OF CARIES-IMMUNE HUMAN BEINGS

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DENTAL CARIES is a bacterial disease initiated by microorganisms living, in the strictest sense, outside the body of the host. The environmental conditions within the oral cavity do not resemble those found on any other external surface of the body, for the oral flora are subject to influence by saliva, presence of concentrated food material, and the activities of the organisms themselves. Dental caries is not an example of obligate parasitism; the injuries sustained by the teeth are only indirect results of rather ordinary courses of bacterial metabolism.

There is little resemblance between dental caries and other bacterial diseases, such as diphtheria or meningitis, except that the etiologic agents are bacteria.[1] For this reason, it is difficult to apply the classic principles of immunology to the study of dental caries yet, in dental research, the concept of host resistance to caries has become accepted. One recognizes varying degrees of susceptibility to this disease in human beings, ranging from rampant attack of caries to complete, continuous resistance. The term "caries-immune" has been applied to human beings exhibiting, after reaching adulthood, such complete resistance. It is obviously futile to attempt to ascertain immunity in very young children where posteruptive exposure of teeth to the oral environment may not have been of sufficient duration for the disease to develop into clinical manifestations.

There is at present little justification for the use of the term "immunity" with reference to dental caries, for specific host responses or defenses have not been clearly demonstrated. Since, however, the etiology of caries is apparently nonspecific, such demonstrations may be limited to nonspecific factors. The difference between a highly caries-resistant person and one who is immune may lie in the quantitative measure of such nonspecific factors in the oral cavity, or possibly in a qualitative lack of an "immune factor" in those who exhibit any degree of susceptibility.

In a search of the literature pertinent to caries immunity, confusions occasionally arise from inadequate descriptions. Individuals have been defined as "caries-free" in instances where there are no active observable lesions, and the same term has been applied to individuals with no open or filled carious lesions. We propose that the term "caries-immune" be reserved for those adult persons having no indication of dental caries, past or present, and that the

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term "caries-free" apply only to those having no demonstrable active carious lesions at the time of examination. Restorations of carious lesions are acceptable in persons of the latter definition, but not the former.

Within the above definition of caries immunity, differences have been shown between caries-immune and susceptible human beings. Lactobacilli have been found absent or only in low numbers in caries-immune saliva by a number of investigators.[2-8] Bunting and Rickert[9] found higher salivary calcium in immune individuals. Fosdick and Campaigne[10] reported a greater carbon dioxide capacity in immune saliva. Krasnow and Oblatt[11] found greater amounts of salivary protein in immune subjects as compared to those caries susceptible, and Orban and Weinmann[12] reported more of a proteolytic enzyme in immune salivas. Grove and Grove[13] found a higher salivary ammonia content in caries-immune individuals, and they, as well as Kesel, O'Donnell and Kirch[14] regarded this difference in ammonia content as the immunizing factor. Green, Dodd, and Radike[15] reported an association of tryptophane or similar substances with saliva from caries-immune subjects. McPhee,[16] using blood sera in immunologic reactions, found higher concentrations of precepitins against carious dentin in caries-immune as compared to caries-susceptible human beings. Williams[17] also found differences of this order, in studying serum precipitins directed against oral lactobacilli. Hill, Matt, and Pearlman[18] have isolated a chemical entity inhibitory to lactobacilli, which is found in greater amount in saliva from caries-immune as compared to caries-susceptible individuals.

These observations have indicated chemical and biologic differences in the saliva from caries-immune and caries-susceptible persons. The reason for caries immunity is unclear, yet it is apparent that factors are operating in immune saliva which help maintain that condition of resistance. Further investigations may disclose whether the differences between immunity and other degrees of resistance are qualitative or quantitative.

The purpose of this study is to observe and define some characteristics of a group of caries-immune human beings. The observations made were both clinical and bacteriologic, and in most cases have been extended over periods of many months.

MATERIALS AND METHODS

Most of the 89 human beings observed in this survey were college students, with some older individuals as indicated by the age range of 17 to 54 years. Sixty-five per cent were males. Whenever possible, each person was examined clinically at least once a year, and salivary lactobacilli counts were made 2 or 3 times per year.

All dental examinations were performed by the same dentist (P. R. W.), following a complete prophylaxis by a dental hygienist. The teeth were examined and charted with the aid of an adequate light, mouth mirror, and explorer. The visual examinations were supplemented by bite-wing x-ray examinations, and complete intra-oral x-ray examinations were made only when the examiner thought it necessary. Any evidence of past or present caries was sufficient to exclude a person from the survey.

The information obtained in the clinical examination was used by the examiner to assign each caries-immune person to one of 3 clinical categories descriptive of the dental condition:

Category 1. Absence of carious lesions, no unexplained missing teeth, no stains in anatomic pits and fissures, no grooves that can catch an explorer point, negative bite-wing radiographs.

Category 2. Absence of carious lesions, no unexplained missing teeth, no grooves that can catch an explorer point, negative bite-wing radiographs, stains present in anatomic grooves, pits, and fissures.

Category 3. Absence of carious lesions, no unexplained missing teeth, deep occlusal fissures that can catch an explorer point, but from which no carious material can be expressed. X-ray pictures may appear positive due to hypocalcification, but on mechanical separation of the teeth, positive diagnosis of caries in the suspected areas is not possible.

Each person was questioned concerning dietary habits, residence in areas having fluoridated water, and occurrence of other caries-immune individuals among blood relations. Such information cannot be regarded as absolutely reliable, and is reported in that light.

Salivary lactobacilli counts were made following the method of Permar and Kitchin,[19] noting the following exceptions. Saliva samples were collected at any time more than one hour after a meal, not before breakfast. Saliva was not diluted before plating on tomato juice agar, 0.1 ml. being spread on the plates. Aerobic and anaerobic cultivation was made of replicate plates. The colony types of lactobacilli which were counted have been described in a previous report.[8]

RESULTS AND DISCUSSION

In the 3 years since this study was initiated, 89 human beings whose ages ranged from 17 to 54 years have been examined clinically and bacteriologically. An even greater number of restoration-free persons was rejected at first examination. A summary of some characteristics of these 89 persons is presented in Table I. It may be seen that, in observations related to enamel hypoplasia and patient history, no clear differences in the 3 groups of persons are evident. The data on salivary lactobacilli counts show that zero or low counts were most common in all the immune subjects. This is in agreement with the findings of other investigators. The only apparent difference between persons in the 3 clinical categories seems to be in occurrence of what we have termed the "immune pattern of salivary lactobacilli." This pattern has either a zero count, or if salivary lactobacilli are found, less than 1,000 per ml. of saliva are observed and these are of smooth colony morphology. This pattern has been found by us to represent the most common distribution of salivary lactobacilli in the caries-immune subjects studied. Since the clinical differences between the 3 categories of immune subjects are slight, even to a trained examiner, it is interesting that even slight nonsignificant but consistent differences in salivary lactobacillus flora are apparent between the first two compared to the third group.

TABLE I

CHARACTERISTICS OF THREE CLINICAL GROUPS OF CARIES-IMMUNE HUMAN BEINGS

OBSERVATIONS	CLINICAL CATEGORIES I	II	III
Number of subjects in group	15	43	31
Male	10	29	19
Female	5	14	12
Age range (years)	18-30	17-54	17-47
Subjects having hypoplastic enamel	8	19	9
Subjects reporting diet restrictions	1	1	3
Subjects reporting immune relatives	3	7	2
Subjects reporting residence in an optimal fluoride area	0	1	1
SALIVARY LACTOBACILLI COUNTS			
Total number of counts in group	50	141	95
Total number of zero counts in group	24 (50%)	82 (59%)	43 (45%)
Total number of counts in "immune pattern"	37 (74%)	112 (79%)	58 (61%)
Subjects having only zero counts	4 (26%)	17 (39%)	7 (22%)
Subjects having "immune pattern"	10 (66%)	25 (59%)	11 (39%)
Subjects having 1 or more counts greater than 5,000	3 (20%)	18 (18%)	8 (22%)

During the period of this investigation, 5 persons accepted into the program developed dental caries while under our observation. Some pertinent data concerning these subjects are summarized in Table II. None of the 5 individuals were originally graded in Category 1, reserved for those with most nearly anatomically perfect teeth. It is clear that the occurrence of the "immune pattern of salivary lactobacilli" was considerably less frequent in these than in persons not known to have developed caries (Table I). In the 5 persons who developed caries, the repeated occurrence of high salivary lactobacilli counts, and especially the presence of rough colony lactobacilli in these, appears to have served as an indicator of impending clinical caries.

TABLE II

CHARACTERISTICS OF PREVIOUSLY CARIES-IMMUNE HUMAN BEINGS WHO DEVELOPED CARIES UNDER OBSERVATION

SUBJECT	CLINICAL CATEGORY ORIGINALLY ASSIGNED	NO. OF MONTHS UNDER OBSERVATION PRIOR TO DETECTION OF CARIES	NO. OF SALIVARY LACTOBACILLUS COUNTS MADE IN PRE-CARIOUS PERIOD	NO. OF SALIVARY LACTOBACILLUS COUNTS IN "IMMUNE PATTERN"*	NO. OF SALIVARY LACTOBACILLUS COUNTS GREATER THAN 5,000
D. C.	III	13	4	1	2
A. C.	II	6	4	0	3
J. L.	III	24	4	3	0
A. P.	II	17	8	1	3
C. R.	III	19	5	1	2

*See text for description.

These data are in agreement with the findings of other investigators reporting low or zero salivary lactobacilli counts in caries-immune human beings. They also indicate that high salivary lactobacilli counts may be associated with preclinical stages of caries activity in persons who are apparently caries-immune.

SUMMARY

Eighty-nine caries-immune human beings were observed clinically and bacteriologically during a 3-year period. These were divided into 3 clinical categories based on the clinical anatomic condition of the teeth. Bacteriologic examination showed that low or zero salivary lactobacilli counts were common in most of the immune individuals, but no striking bacteriologic differences were found among the 3 clinical groups. Five individuals who developed caries under observation were found to exhibit differences in salivary lactobacilli from those who did not develop dental caries.

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THE RELATION OF STREPTOCOCCI, LACTOBACILLI, AND THE GENERAL ORAL AND FECAL FLORA TO THE PROGRESSION OF DENTAL CARIES IN THE HAMSTER

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ALTHOUGH the Syrian hamster (*Mesocricetus auratus*) has served as an experimental animal in the study of dental caries since 1942,[1] our knowledge concerning the ecology of the oral bacteria of this animal is limited. The oral bacterial flora of the hamster was studied by Orland[2] in 179 animals maintained on various adequate and deficient diets. The caries-susceptible animals already had considerable caries experience. When fecal cultures were made from several of the hamsters to determine whether any of the oral forms occurred in the intestine, the results were equivocal.

Helman and Mitchell[3] collected hamster saliva using a method similar to that of Orland.[2] No correlation was found between caries experience and salivary lactobacillus counts of 40 adult hamsters maintained on 3 different diets. A significant degree of association was found between the numbers of lactobacilli and coliforms. The investigators suggested that intestinal lactobacilli introduced into the mouth of the hamster through coprophagy may affect the true lactobacillus count. In a second group of animals, the results were inconsistent.

Gustafson, Stelling, Abramson, and Brunius,[4] from some comparative observations on the oral bacterial flora of 4 groups of animals maintained on diets with 0, 8, 16, and 48 per cent sucrose, found that the animals on the cariogenic diets had greater mean numbers of lactobacilli and streptococci than the animals on the noncariogenic diet (0 per cent sucrose).

Keyes,[5] and Frisbie and Nuckolls[6] made histopathologic studies of molars which showed intact discolored areas, and found that bacteria penetrated along confined channels to the dentinoenamel junction where they seemed to establish foci of spread.

This paper describes an application of the Johansen technic[7, 8] to obtain salivary samples from the hamster for quantitative bacteriologic study; presents data concerning repeated and progressive oral lactobacillus and streptococcal populations in animals on certain cariogenic and noncariogenic diets;

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relates these organisms to the total salivary and fecal flora; and attempts to correlate the streptococcal and lactobacillus populations with the initiation and progress of dental caries in the hamster.

EXPERIMENTAL METHODS

Composition of the diets: The noncariogenic diet (Diet 1) consisted of 46 per cent powdered whole milk, 46 per cent whole wheat flour, 6.5 per cent ground alfalfa, and 1.5 per cent sodium chloride. The cariogenic diet (Diet 2) consisted of 30 per cent powdered whole milk, 30 per cent whole wheat flour, 20 per cent cornstarch, 15 per cent sucrose, 4 per cent ground alfalfa, and 1 per cent sodium chloride. The fluorine content of Diet 1 was 0.32 ppm; that of Diet 2 was 0.47 ppm; and that of the tap water was 0.12 ppm. These diets were selected because previous experience had shown that Diet 2 was a satisfactory cariogenic diet and that Diet 1 served adequately as a noncariogenic control diet.

Selection of the animals and experimental conditions: Sixty-four caries-susceptible hamsters from the University of Rochester stock colony were selected for the experiment. Before weaning, these animals had access to the noncariogenic Diet 1 which was furnished the mothers as a stock diet and were housed in breeding cages containing a bottom covering of wood shavings. At 25 days of age, 33 males and 31 females from 12 litters were weaned, and placed into 2 groups of littermates. Group I was maintained on Diet 1. Group II was fed Diet 2. During the entire experimental period, the animals were isolated and housed individually in screen bottom cages whose side walls and backs were made of galvanized solid sheet metal. The 2 groups of animals were kept on opposite sides of a double rack with no opportunity for physical contact. Food cups and water bottles were marked indelibly for the individual cages and were used exclusively by individual animals. The experimental diets and tap water were available ad libitum. The animals were weighed periodically and their weight gains were recorded.

Technic of salivary sampling: The immobilizing instrument described by Johansen[7, 8] was modified by removing the rubber covering on the mandibular stabilizer. The immobilizing instrument and all surgical instruments used in sampling procedures and oral diagnostic examinations were sterilized before use by immersion in 70 per cent alcohol and subsequent flaming in the Bunsen burner.

All samples were procured while observing the appropriate oral area with a wide-field microscope having a magnification of ×24. The saliva was aspirated into a sterile capillary pipette attached to a sterile assembly as shown in Fig. 1. The blood-dilution pipette in the middle was plugged with sterile absorbent cotton to assure against contamination of the sample from the operator's saliva. The sterile rubber tube holding the capillary pipette and the sterile pipette itself were changed with each sampling.

Mixed samples were taken from each of the 4 quadrants of the mouth: along the buccal aspect of the maxillary molar dentition on both right and left sides and along the areas lingual to the mandibular molar dentition on both sides. The saliva and available tooth debris were aspirated by creating negative pressure in the pipette while moving it slowly forward from the third molar region to the first molar region. After obtaining a composite sample from the 4 areas, the specimen was blown out slowly so that a minute drop formed at the tip of the pipette. No mixing other than that which occurred in the alternate aspiration and return to atmospheric pressure was attempted. A loopful was taken from this drop with a sterile platinum bacteriologic loop whose volume was 55×10^{-5} ml. The same loop was used throughout the study.

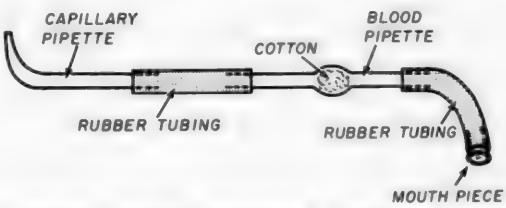

Fig. 1.—Pipette assembly employed in sampling of oral areas.

The loop sample was inoculated by agitating it for 10 seconds in 5 ml. of 0.03 per cent yeast extract solution. Immediately thereafter a drop of saliva was deposited from the capillary pipette on a clean glass slide to make a smear for microscopic examination. The presence of food or fecal material in the mouth was recorded at each salivary sampling.

Oral diagnostic examination: After procuring salivary samples for bacterial studies at 8, 17, 24, 32, 46, 65, 81, 109, 155, and 228 experimental days, the live unanesthetized animals were given a detailed oral examination according to the technic previously cited.[7, 8] In the early phases of the study the progress of eruption of the teeth and the condition of the molars and the surrounding soft tissues were noted. Dental caries, periodontal disease, and debris accumulations were observed and the progression of periodontal disease and dental caries were recorded according to the Johansen[8] modification of the Keyes[9] scoring method. Only the data concerning dental caries will be presented in this paper.

Bacteriologic methods: A standard loopful of saliva was transferred by means of the standardized technic to 5 ml. of 0.03 per cent yeast extract solution. This is mentioned subsequently as the $\frac{1}{5}$ dilution. This holding medium was selected because in preliminary experiments the populations of the microorganisms to be studied did not change beyond the limits of experimental error during a 4-hour holding period at room temperature. Aliquots from the yeast extract were further diluted to $\frac{1}{50}$ and $\frac{1}{500}$, using distilled water as the diluent. The $\frac{1}{5}$ and $\frac{1}{50}$ dilutions were pour-plated in SL agar,[10] modified by the

addition of 0.5 per cent L-arabinose and 1 per cent sucrose, and by the reduction of the glucose content to 1 per cent. This modification makes an improved medium and is now standard for the primary isolation of lactobacilli in the authors' laboratory. Plates were incubated for 3 days at 35° C. and the lactobacillus colonies were then carefully counted.

For the enumeration of streptococci, plates were poured at dilutions of $\frac{1}{50}$ and $\frac{1}{500}$ using the following medium: dehydrated brain heart infusion (Difco), 35.5 Gm.; sodium azide, 0.1 Gm.; crystal violet, $\frac{1}{500,000}$; agar, 1.5 per cent; and H_2O to 1 L. Five per cent of defibrinated sheep blood was added to the melted agar at 45° C. immediately before use. The streptococcal plates were incubated for 48 hours at 35° C. Each plate was examined carefully with the aid of a wide-field microscope, using both reflected and transmitted light. The alpha, beta, and gamma types were counted differentially and the total number of all types of streptococci was also recorded.

All media were prepared the day preceding the sampling.

The populations of lactobacilli and streptococci were expressed as numbers per standard loop of saliva. To express the populations as numbers per milliliter of saliva, the numbers of bacteria per loop should be multiplied by the factor 1,800.

The smears of saliva, which had been made at each sampling, were stained by the Burke modification of Gram's method. These smears were examined microscopically and the qualitative distribution of the various groups of microorganisms was recorded.

Since the coprophagous habit of the hamster might affect the bacterial ecology of the mouth, a study was made of the lactobacillus, streptococcus, and coliform populations of the feces. In addition to the final oral sampling, on the 228 day of the experiment, a sampling of feces was also obtained. Dorsal stroking of the animal anteriorly to the tail insert induced the animal to void feces which were collected directly in a sterile bottle. The feces were weighed to the nearest centigram and the fecal samples were introduced into a screw-cap bottle containing 100 ml. of sterile distilled water. The bottles were shaken vigorously by machine for 20 minutes. This treatment comminuted the fecal particles sufficiently to yield maximal counts of bacteria. The dispersed fecal suspensions were further diluted $\frac{1}{10}$, $\frac{1}{100}$, and $\frac{1}{1,000}$ and the numbers of bacteria per gram were calculated from the known fractional weights of the original fecal samples according to the following formula:

$$\text{no./gram} = \frac{1,000}{\text{mg. of sample}} \times \text{final dilution.}$$

Breed smears were made from the fecal suspensions for microscopic examination. When the animals were sacrificed after the 228 day of experiment, the internal organs were examined grossly, the cecum punctured, and smears made of the cecal contents for microscopic study.

Before weaning, the types of oral lactobacilli present were isolated and studied in great detail. Eight days after the animals were weaned and given

the experimental diets, the oral lactobacilli were again isolated and typed. The representative lactobacilli present in the fecal sampling were also isolated and studied. The results of these lactobacillus typings will be discussed only in broad, general outlines.

RESULTS

Nutritional state and general health of the animals: One female receiving Diet 1, and 1 male and 1 female fed Diet 2 died during the experimental period of 228 days. The rate of weight gain and the final weights (60 to 70 Gm.) were nearly similar for the animals on the 2 diets. The weight gains were not so great as those noted on more recently improved diets and other circumstances. However, in addition to the probable partial inadequacy of the diets, the weight gains were probably limited also by the animals' voluntary restriction of food intake while being maintained in solitary confinement. Other animals caged in groups no greater than 4 repeatedly gained 40 per cent more weight after 60 days on Diet 1 than the hamsters in this experiment.[11] Examination of the internal organs at autopsy did not reveal any gross signs of pathosis.

Relation of the bacteriologic data to the initiation and progress of caries: Summaries of the results of these studies are presented in Figs. 2 to 5. The numbers of oral lactobacilli and streptococci at each sampling and the progressive caries scores are plotted semilogarithmically against the number of days on the diets. The first bacteriologic determination was performed 5 days before the animals were weaned, separated into 2 groups and placed on the experimental diets. Hence the term "–5 pre-diet days" appears in the lower left corner of the figures.

Initially the mean streptococcal counts were considerably lower than the mean numbers of lactobacilli in all groups of weanling animals. However, the streptococcus populations increased steadily and greatly throughout the experiment. Regardless of diet, the final mean numbers of streptococci increased approximately 30 fold. The curves representing the numbers of streptococci in females or in the males on the 2 diets can be nearly superimposed. Statistical evaluation of the mean differences using Student's t test indicates that there was no significant difference in streptococcal populations on either diet.

While the streptococcal counts increased, in general the mean numbers of lactobacilli decreased and fluctuated widely between individual runs. It may be remembered that the final mean streptococcal count for all animals increased 3,000 per cent as compared to the initial count. The lactobacilli, on the other hand, decreased approximately 50 per cent.

Obviously, one of the purposes of this study was to determine the relationship of the lactobacilli to dental caries in the hamster. The data in Fig. 3 for the females on the cariogenic diet (Diet 2) show that the general trend of lactobacillus populations was decreasing immediately before caries was detected. Once caries experience was noted the lactobacilli tended to increase within a range of wide fluctuation; but, Fig. 2 shows that the same trends were present in the females on the noncariogenic diet (Diet 1). Although the

shift of bacterial populations was not different in the case of the 2 diets, the caries experience was markedly different. Only 2 in a total of 15 females (14 per cent) had any gross evidence of caries on Diet 1, while 13 of 14 females

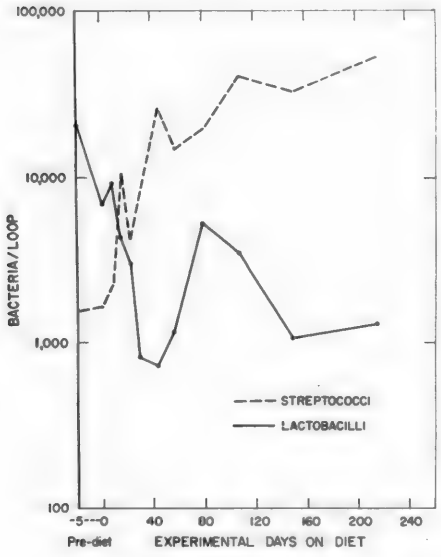

Fig. 2.

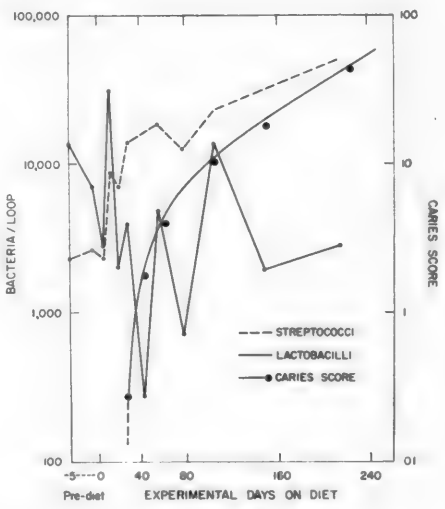

Fig. 3.

Fig. 2.—Mean counts of streptococci and lactobacilli in females on Diet 1.
Fig. 3.—Mean counts of streptococci and lactobacilli and mean caries scores in females on Diet 2.

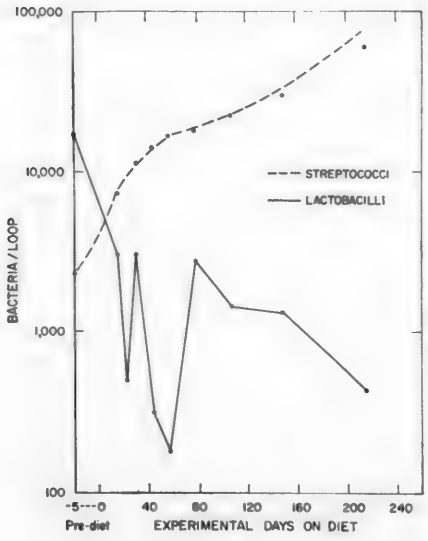

Fig. 4.

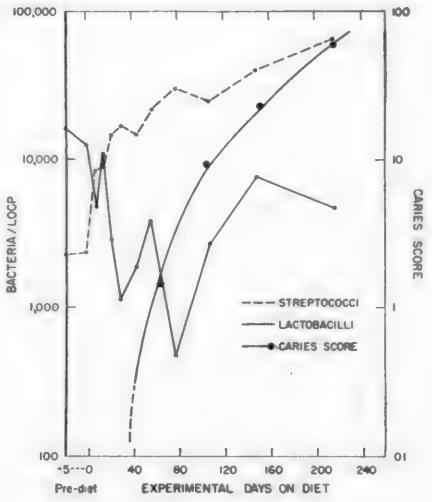

Fig. 5.

Fig. 4.—Mean counts of streptococci and lactobacilli in males on Diet 1.
Fig. 5.—Mean counts of streptococci and lactobacilli and mean caries scores in males on Diet 2.

(93 per cent) developed caries on Diet 2. Furthermore, the final mean score on Diet 1 was negligible (0.04) whereas the comparable score on Diet 2 was 43.

In the case of the males only 2 of 16 (13 per cent) animals on Diet 1 were diagnosed as carious whereas all 16 hamsters (100 per cent) developed caries on Diet 2. The respective final mean scores were 0.06 and 59. As in the case of the females the lactobacillus populations decreased after weaning. But, unlike the females the numbers of lactobacilli tended (except for 2 determinations) to be greater on Diet 2 but only long after caries was first diagnosed. Statistical evaluation by means of the t test confirmed that these differences in the eighth, eleventh and twelfth runs were significantly different. However, in the ninth run the lactobacilli were significantly more numerous on Diet 1 rather than on Diet 2. Furthermore, it is difficult to explain why the differences in the intermediate tenth run were not significant. The results of the statistical studies are presented in Tables I and II.

TABLE I
STATISTICAL EVALUATION OF THE MEAN DIFFERENCES IN POPULATION OF LACTOBACILLI ON THE EXPERIMENTAL DIETS

RUN	MALES		FEMALES	
	t	P	t	P
3	0.024	0.9 -1.0	−1.099	0.2-0.3
4	0.721	0.4 -0.5	0.895	0.3-0.4
5	1.296	0.2 -0.3	−0.338	0.7-0.8
6	−0.166	0.8 -0.9	1.190	0.2-0.3
7	0.994	0.3	−0.579	0.5-0.6
8	3.255	0.01-0.001	0.999	0.3-0.4
9	−1.996	0.05	−0.710	0.4-0.5
10	0.426	0.6 -0.7	0.582	0.5-0.6
11	2.359	0.05-0.02	0.488	0.6-0.7
12	2.923	0.01-0.001	0.673	0.5-0.6

$$(\bar{x}_1 - \bar{x}_2) = \sqrt{\frac{(N_1 + N_2)\ (x_1^2 + x_2^2)}{N_1N_2(N_1 - 1) + (N_2 - 1)}}$$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\bar{x}_1 - \bar{x}_2}$$

$(\bar{x}_1 - \bar{x}_2)$ = difference of the standard error of the means

$\bar{x}$ = mean

N = no. samples

TABLE II
STATISTICAL EVALUATION OF THE MEAN DIFFERENCES IN POPULATIONS OF STREPTOCOCCI ON THE EXPERIMENTAL DIETS

RUN	MALES		FEMALES	
	t	P	t	P
3	0.569	.5-.6	0.020	.9-1
4	0.334	.7-.8	−0.185	.8-.9
5	0.484	.6-.7	0.553	.6
6	0.453	.6-.7	0.467	.6-.7
7	0.054	.9-1	−0.485	.6-.7
8	0.344	.7-.8	0.247	.8
9	0.721	.4-.5	−0.483	.6-.7
10	−0.780	.4-.5	−0.499	.6-.7
11	0.408	.6-.7	−0.079	.9-1
12	0.088	.9-1	−0.218	.8-.9

It seems profitable to present the data for a small number of individual cases illustrating the chief types of variance. Fig. 6 depicts the trends in a female which developed caries early in the experiment. One can see that the numbers of lactobacilli nearly paralleled the increase in progressive caries scores. Fig. 7 illustrates the case of an animal which never developed caries on the cariogenic Diet 2. Lactobacilli were absent in nearly every determination after 25 days of the experiment. These 2 instances might be accepted as indicating good correlation between the presence of lactobacilli and caries progression. However, Fig. 8 indicates an opposite situation in which the lactobacillus counts were negative or nearly so while caries was progressing. Fig. 9 shows that lactobacilli were often present in appreciable numbers while caries was nonexistent in this animal. Furthermore, during a period of 50 days, the lactobacillus counts in this animal were nearly twice as great as in the animal in Fig. 6 which developed caries early within this specified period and in which caries was progressing rapidly and severely.

Studies of the fecal flora: The mean numbers and population range of the fecal coliforms, lactobacilli, and streptococci are shown in Table III. Of the 3 groups of microorganisms the lactobacilli were most abundant, the streptococci less so, and the coliforms were present in relatively low numbers. For example, 43.2 and 21.3 per cent of the samples from Diets 1 and 2, respectively, yielded negative counts for coliform bacteria. In contrast, there were no samples in which the lactobacillus count was less than 1,990,000 per gram of feces. Also, there were no cases in which the streptococci were present at numbers less than 650,000 per gram.

TABLE III
MEAN NUMBERS AND POPULATION RANGE OF CERTAIN FECAL BACTERIA

RANGE (NO. PER GRAM)	COLIFORMS		LACTOBACILLI		STREPTOCOCCI	
	DIET 1	DIET 2	DIET 1	DIET 2	DIET 1	DIET 2
	% OF SAMPLES		% OF SAMPLES		% OF SAMPLES	
0	43.2	21.3	0	0	0	0
10^5	6.7	7.2	0	0	0	0
10^5-10^6	20.0	10.7	0	0	6.7	0
10^6-10^7	16.7	32.3	10.7	10.7	40.0	21.4
10^7-10^8	6.7	10.7	53.6	35.7	46.6	57.2
10^8-10^9	6.7	17.8	32.1	46.5	6.7	21.4
10^9	0	0	3.6	7.1	0	0
MEAN	NO. PER GRAM		NO. PER GRAM		NO. PER GRAM	
	15.7×10^5	46.9×10^5	182×10^6	331×10^6	29.6×10^6	71.6×10^6

The mean numbers of the 3 groups of organisms were greater in the samples from Diet 2 than those from Diet 1 and were present in the ratios of 3.0:1 for the coliforms, 1.8:1 for the lactobacilli, and 2.4:1 for the streptococci. However, statistical treatment of the data using the t test indicated that the means were not significantly different. For the coliforms the P value was 0.3-0.4; for the lactobacilli, $P = 0.3$; and for the streptococci, $P = 0.08$. Only the latter value might be considered suggestively significant.

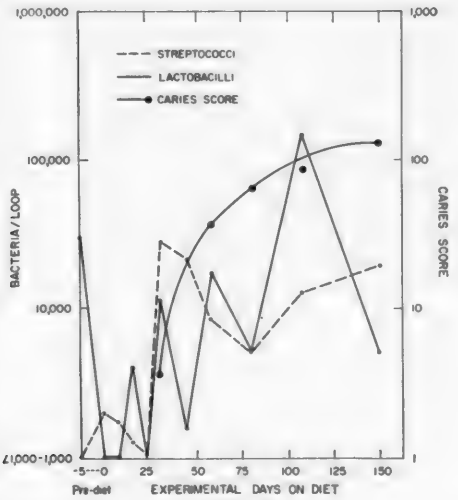

Fig. 6.

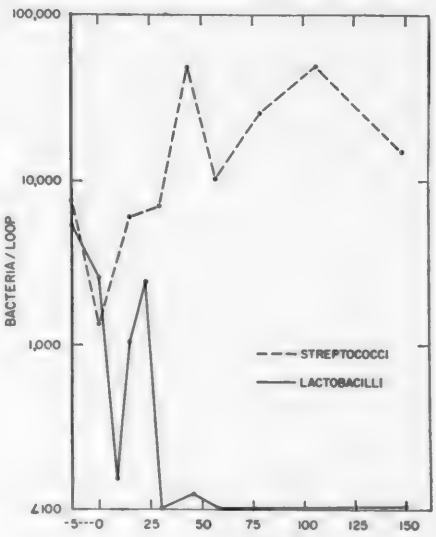

Fig. 7.

Fig. 6.—Mean counts of streptococci and lactobacilli and mean caries scores in an individual female animal which developed caries early on Diet 2.

Fig. 7.—Mean counts of streptococci and lactobacilli in an individual animal which did not develop caries on Diet 2.

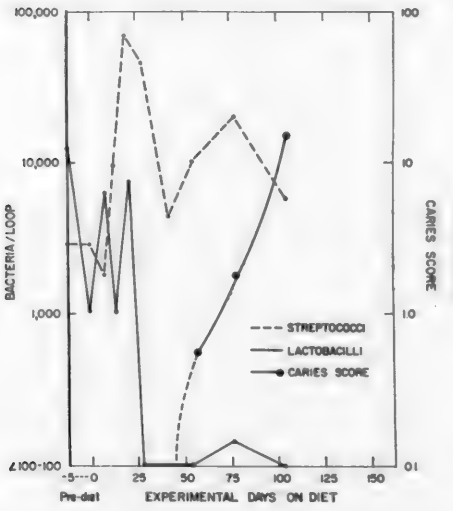

Fig. 8.

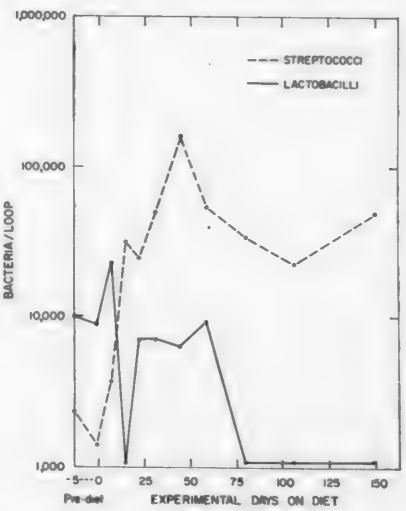

Fig. 9.

Fig. 8.—Mean counts of streptococci and lactobacilli and mean caries scores in an individual animal on Diet 2. Note low or negative lactobacillus counts during the progression of caries.

Fig. 9.—Mean streptococcus and lactobacillus counts in an individual animal on Diet 2. Note absence of caries and high lactobacillus counts during a 50-day period. Compare with Fig. 7, in which the lactobacilli were only half as numerous, and in which caries was progressing rapidly and severely.

While there was no significant difference between the experimental diets in numbers of oral streptococci at any time and the ratio of streptococci to lactobacilli in the oral samples was 24:1, in the fecal samples the lactobacilli were predominant rather than the streptococci and exceeded the streptococci by a ratio of 5:1. Therefore, if coprophagy per se were important in the establishment or maintenance of the oral flora, it is difficult to understand how fecal material which has a preponderance of lactobacilli over streptococci could influence the oral environment so as to establish a widely different oral ecology in which the streptococci were predominant.

Representative colonies of lactobacilli were isolated and the isolates were compared with the previously obtained oral isolates by means of the methods of Rogosa, Wiseman, Mitchell, and Disraely.[12] The fecal lactobacilli were not appreciably different qualitatively from the oral strains. The hamster lactobacilli have been shown by Rogosa and Disraely[13] to be distinctly different from human strains.

Isolations were made from each positive eosin methylene blue plate into brilliant green lactose broth. Surprisingly, only one sample yielded colonies resembling *Escherichia coli*. In all the other cases the original colonies appeared to be *Aerobacter aerogenes*. However, when the isolates were examined in great detail, it was found that none of the strains were members of this species but were rather hitherto undescribed intermediates which were slow lactose fermenters, producing gas after 48 to 96 hours of incubation. Many of the strains showed no evidence of gas production until after 72 hours.

Study of the cecum: The contents of the cecum were not cultured. However, examination of wet mounts and air dried gram-stained smears revealed a teeming population of microorganisms largely different from those of either the oral or fecal environments. Uniquely characteristic of the cecum were a large number of protozoan organisms diagnosed as trichomonads by Wantland.[14] The remaining organisms were chiefly abundant numbers of spirochetes and fusiforms.

Microscopic examination of the oral samples: The gram-stained smears of the oral samples contained large populations of microorganisms. Streptococci were seen generally but only in relatively moderate numbers. Cells resembling lactobacilli were observed infrequently. Fusiform bacteria were present very frequently and often, it seemed, in greater numbers in those animals with severe caries. But also a very great population of very large coccal organisms was nearly always found. These organisms were often pleomorphic, tending to elongate almost as rods and sometimes appearing in chains. They seemed not to be streptococci. Yeasts were detected frequently and spirochetes were also found. Apparently the streptococci and the lactobacilli were only a small fraction of the total oral flora and therefore the great mass of organisms was not cultured at all.

Microscopic examination of the feces: Certain groups of organisms similar to those in the mouth were also present in the feces. Streptococci were seen in about the same proportions as in the oral smears. The large coccal organisms

and fusiforms also were common to both environments. The trichomonads found in the cecum were not observed in either oral or fecal smears. The spirochetes abundant in the cecum were absent or present sporadically in small numbers in fecal smears. A large group of gram-negative rods not present in either the cecum or the mouth, and which may be interpreted to be bacteroides-like organisms, were constantly and greatly in evidence. It is highly unlikely that these organisms were coliforms since the numbers seen on smears, even if 90 per cent of the gram-negative organisms were dead, are not consistent with the cultural findings for the coliforms. Furthermore, the morphologic characteristics were widely dissimilar. Organisms which could be construed as lactobacilli were present in relatively very small numbers. It is clear, then, that most of the fecal flora was not cultured by any of the technics employed in this study.

DISCUSSION

With 2 litter-mated groups of animals on rather closely related diets, one cariogenic and the other essentially noncariogenic, there was a sufficiently high number of animals with and without the disease. Sixteen (100 per cent) of the 16 males and 13 (93 per cent) of the 14 females on the cariogenic diet developed gross evidence of caries during their lives while only 2 (13 per cent) of 16 males and 2 (14 per cent) of 15 females on the noncariogenic diet had lesions which were detectable in the live animal.

After sacrifice, careful examination of the cleaned skulls of the animals from the noncariogenic diet revealed that 7 additional males and 6 females had root surface lesions. These lesions were, however, extremely small and did not materially change the previously determined negligible mean total caries scores as already assessed in the live animal. A comparison of total scores, including root surface lesions expressed in Keyes[9] scores, shows that the mean score for the males on the cariogenic diet was approximately 150 times the score of the males on the noncariogenic diet. For the females the respective relationship was 100 fold.

An important limitation in previous studies of the relation of bacteria to the initiation and progress of caries in experimental animals has been the lack of convenient technics for simultaneous bacteriologic sampling and dental examinations. The results of a single dental examination may be only remotely related in time to the initial or early bacteriology of a previously sound tooth surface. This relationship may be even more remote in the case of salivary samples. This limitation has been overcome in the present endeavor which permitted the dynamic aspects of tooth destruction by the caries process to be studied by means of repeated oral examinations of the same animal at successive intervals. This procedure has given much information as to the relative susceptibility of a tooth, revealed more accurately the point of origin, and thus the pattern of destruction of indivdiual molars was established with considerable accuracy. The guesswork which often necessarily accompanies similar interpretations based on the study of sacrificed animals at the end of an experimental period was largely eliminated.

Three general patterns of the progression of caries were found: (1) early initiation most frequently was followed by rapid progression and high final scores; (2) later initiation, however, also frequently resulting in rapid progression with the final score determined by the length of the experimental period; and (3) other late lesions progressing slowly with a low final score. Thus, 2 lesions of equal size might have resulted from different intensities of the caries process. Correlative bacteriologic results and caries scores for each of these 3 general patterns of caries progression have been presented in Figs. 6 and 8.

The destruction of individual molars sometimes occurred in a steplike manner while in other cases it was nearly linear over several examinations. The reason for this variation is most likely to be found in differences of intensity of the caries process and location of sites of initial attack. Each tooth has its own more or less individual pattern of destruction. The lesions have their point of origin on the enamel or at the cemento-enamel junction. The mode of attack of individual teeth and surfaces of the animals in this study has been adequately described by Johansen.[8, 11] He has discussed in great detail the extensive loss in tooth structure which is sufficiently striking to be detected early in the live animal and which justifies the caries evaluation technic used in this study.

It is now of interest to recall that in this study no significant difference in caries experience was noted between the two sexes as judged from the final scores. This finding is in agreement with one other study and disagrees with 5 others from this laboratory.[11]

There is much that is unknown as to how the carious lesions may affect the bacteriology of the oral cavity and vice versa. The susceptible molar areas are extremely small as compared to the entire oral cavity. It seems reasonable to assume that the teeth and gingival crevices provide the best areas for relatively stationary masses of bacteria to develop. But it does not necessarily follow that the whole bacterial flora of the mouth should be determined by these sites or by organisms from the cavities in carious teeth, since very frequently such cavities have been found to be plugged with temporary or semipermanent accumulations of debris or fecal matter.

The previously presented evidence indicates that the streptococci and lactobacilli are only a limited portion of the salivary and fecal flora. Tentative evidence has been accumulated which indicates that fecal contamination, per se, may not determine the salivary flora to the extent previously widely assumed. Rosebury, Foley, Greenberg, and Pollack[15] have presented some evidence from the rat that points in this direction. The mouth has an efficient clearing mechanism so that organisms from one environment do not necessarily become established in another. For instance, *Serratia marcescens, Sarcina lutea,* and certain yeasts are not implantable in the human mouth[16-23] and lactobacilli are not found in the nasopharyngeal region in man.[24] However, only

after more knowledge is gained concerning the total flora of both the mouth and feces will it be possible to arrive at more definitive conclusions as to the ecologic relations of the various anatomic regions.

The authors appreciate the importance of bacteriologic studies of tooth surfaces and carious lesions. However, early attempts to devise workable technics for the quantitative bacteriologic sampling of discrete tooth surface areas were not successful. More recent experiments indicate the possibility of succeeding in this direction. If this can be done, if the dynamic progress of caries be also determined simultaneously, and if a greater variety of selective media becomes available for more complete studies of the total oral and fecal flora, vastly improved knowledge of the mouth in health and disease will be inevitable.

SUMMARY

1. A technic for repeated bacteriologic samplings of hamster saliva and correlative dental examinations has been described.

2. No positive relationship was found between salivary streptococci and dental caries in either male or female hamsters.

3. No correlation was observed between salivary lactobacilli and dental caries in females.

4. In male hamsters a positive relationship was noted between lactobacilli and dental caries, but only after very extensive development of lesions.

5. The final mean caries scores of males and females in this study were not significantly different.

6. The ecologic relations of the streptococci and lactobacilli were widely different in the mouth as compared to the feces.

7. Microscopic examination of oral and fecal smears indicated that the streptococci and lactobacilli were a relatively small part of the total flora and that a great percentage of the organisms, such as fusiforms, bacteroides, large coccal organisms other than streptococci, etc., have not been cultured.

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THE EFFECT OF SEX HORMONES ON DENTAL CARIES IN DESALIVATED RATS

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ORCHIECTOMY consistently produces a significant reduction in dental caries in the rat.[1, 2] The result of ovariectomy is more variable; however, some studies indicating an increase[1, 2] and others a decrease in dental caries.[3, 4] Since several workers have suggested that the sex hormones are important in the maintenance of normal salivary gland function[5, 6] and histology,[7-9] it is reasonable to suppose that the salivary glands may be the agents through which the sex hormones alter the incidence of dental caries. Castration may retain its anticariogenic action even in the absence of the major salivary glands,[3] and so it seemed important to investigate the effects of sex hormones on dental caries experience in the absence of the major salivary glands.

EXPERIMENTAL

A total of 160 weanling Sprague-Dawley strain rats were divided equally according to sex and initial weight into 4 experimental groups. Group 1 was castrated and, in addition, received daily intramuscular injections of the respective sex hormones. The males received 250 μg per rat per day of testosterone propionate in aqueous suspension while the females were injected daily with 50 μg per rat of estradiol dipropionate in croton oil. Group 2 was castrated and, in addition, the major salivary glands were removed.[3] These animals also received daily hormone injections, the males getting testosterone and the females estradiol at the same levels received by the animals in Group 1. Group 3 was both castrated and "salivariadenectomized." The males in this group received estradiol and the females testosterone at the same levels as received by Groups 1 and 2. Group 4 was unoperated, received no hormone injections, and served as controls. The animals were housed in pairs in raised screen cages in a temperature controlled room. Food and water were available ad libitum. All the animals received a coarse corn cariogenic diet* and fluorine-low water for 140 days at the end of which time the animals were sacrificed by ether and the heads removed and examined for dental caries by methods previously described.[10]

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*The composition of the diet used in this study was as follows: yellow corn grits, 52.7%; ground yellow whole corn, 11.3%; powdered whole milk, 30.0%; alfalfa, 4.8%; iodized sodium chloride, 1.0% and irradiated yeast, 0.2%.

DATA AND DISCUSSION

Both ovariectomy and orchiectomy resulted in a significant reduction in the dental caries incidence.* In this study the injection of male or female hormone into the respective animals (Table I) eliminated the anticariogenic effect of castration and restored the caries pattern essentially to the control level. Although daily food consumption was not measured, food intake does not seem to be a prominent factor in these results since weekly food intake measurements indicated that the castrated animals ate at least as much food as the control animals and the food intake of the hormone injected animals was only slightly less than the controls.

TABLE I

EFFECT OF SEX HORMONES ON DENTAL CARIES INCIDENCE IN DESALIVATED RATS

	NO. ANIMALS PER GROUP	FINAL WT. (GM.)	MEAN NO. CARIOUS LESIONS	PER CENT CHANGE	MOLARS AFFECTED	EXTENT
			Males			
Castrate plus testosterone	14	338	8.5	+12	4.21	2.53
Desalivate and castrate plus testosterone	16	275	12.2	+60	6.12	2.55
Desalivate and castrate plus estradiol	15	208	12.3	+62	6.27	2.69
Control	16	298	7.6	--	4.30	2.40
Castrate*	17	284	5.5	-28	4.20	1.93
Desalivate	17	283	12.9	+69	6.80	2.54
Desalivate and castrate*	16	245	10.1	+33	5.53	2.50
			Females			
Castrate plus estradiol	15	185	8.5	-1	4.40	2.61
Desalivate and castrate plus estradiol	14	171	11.2	+30	5.90	2.72
Desalivate and castrate plus testosterone	16	225	12.2	+42	6.38	2.54
Control	12	206	8.6	--	4.25	2.60
Castrate*	15	254	4.7	-37	3.91	1.92
Desalivate*	15	199	12.6	+47	6.60	2.60
Desalivate and castrate*	15	251	9.9	+15	5.31	2.44

*Data previously reported.[3]

Although salviariadenectomy increased the caries rate by approximately 60 per cent, castration still maintains the effect of reducing the caries rate by approximately 35 per cent in salivariadenectomized animals.[3, 4] This result would suggest that the major salivary glands per se are not important as intermediates in explaining the anticariogenic effect of castration. Moreover, the injection of either sex hormone to castrated and salivariadenectomized rats returned the

*The effect of salivariadenectomy and castration on dental caries has previously been reported[3] and these data appear in Table I for comparative purposes only.

caries incidence to the same degree as that found in salivariadenectomized animals alone. A considerable amount of evidence has been accumulating which links salivary gland function and the incidence of dental caries. For example, Shafer and Muhler indicated a close correlation between the histologic appearance of the submaxillary gland and its proteolytic activity and dental caries incidence. Although several workers have indicated that the sex hormones may control, at least in part, normal salivary gland function,[5-7, 11] the data presented here and in previous reports[3, 4] show that loss of the major salivary glands does not affect either the anticariogenic effect of castration or the cariogenic effect of sex hormone injections. Apparently the salivary glands are not important intermediates in these results.

It is interesting to note that estradiol in these salivariadenectomized animals seemed to have a more pronounced effect on the severity of the lesions produced than testosterone. This confirms previous findings in unoperated animals.[12]

Muhler and Shafer[13] and Bixler, Muhler, and Shafer[14] indicated that the major salivary glands are important in the relationship between the thyroid gland and dental caries. The data presented here, however, suggest that the sex hormones act in a different manner. It seems likely, because of the known metabolic actions of testosterone and estradiol, that, even though these 2 hormones may act on dental caries in a similar fashion, they may exert their effects on caries in the rat in some manner which does not involve the major salivary glands.

SUMMARY

Injection of either estradiol or testosterone into castrated animals restored the dental caries incidence to the control level. Similarly, the injection of these hormones into castrated and salivariadenectomized rats restored the caries incidence to the same high level found in salivariadenectomized rats. These results indicate that the major salivary glands are probably not important intermediates in a sex hormone-dental caries relationship in the rat.

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ENZYMES OF HUMAN SALIVA

II. PAROTID SALIVA TOTAL ESTERASES

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SODIUM beta-naphthylacetate has been employed as a substrate for the demonstration of nonspecific esterases in tissues[1] and for the colorimetric determination of esterases in saliva.[2] Although this substrate is most readily attacked by the esterases of liver and blood serum, it can also be hydrolyzed by the lipases found in pancreatic homogenates.[3] The ability of acetyl- and pseudo-cholinesterases to split β-naphthylacetate has been indicated by studies employing brain homogenate, serum cholinesterase, and red cell membranes.[4] Therefore, in the measurement of the enzymatic hydrolysis of β-naphthylacetate by human saliva the term "total esterase activity" has been employed to encompass activity originating from nonspecific esterases, cholinesterases, and lipases. The results given here represent a partial kinetic characterization of the total esterase activity of human parotid saliva.

METHOD

Chicle-stimulated parotid saliva was collected from a group of normal subjects by means of the Curby parotid cap.[5] All samples were obtained in refrigerated tubes, pooled, and tested immediately after collection. No demonstrable loss of activity occurred in samples refrigerated for 5 days and the enzyme was stable at room temperature for at least 8 hours.

Total esterase activity was measured by the method of Seligman and Nachlas,[6] modified for use with saliva.[2] Veronal buffer (0.2M) was employed to maintain the desired alkaline pH levels with saliva. All pH values reported are those measured in the final incubation mixture containing 2.5 ml. of buffer, 2.5 ml. of substrate, and 3.0 ml. of saliva. 0.5M HCl or 1.0M Na_2CO_3 was added to the assays after incubation to adjust the pH to the optimal range (7.4 to 7.8) for color development. Enzymatically liberated β-naphthol was determined by means of the water insoluble complex which formed on the addition of tetrazotized diorthoanisidine (Diazo Blue B) at alkaline pH. This dye was extracted with ethyl acetate and the color density measured in a Klett photoelectric colorimeter using 540 mμ green filter. All assays of parotid saliva total esterase activity were measured in triplicate. Substrate

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controls for determining the degree of spontaneous hydrolysis under the various test conditions were prepared by incubating 5.0 ml. of buffered substrate, to which saliva was added just prior to coupling with the azo dye.

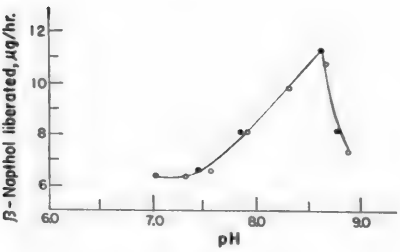

Fig. 1.

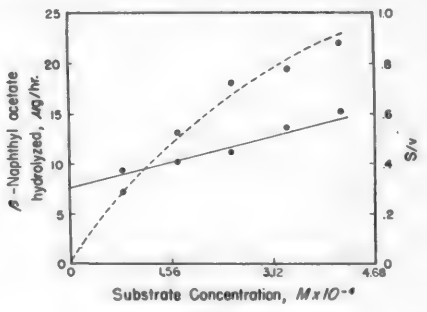

Fig. 2.

Fig. 1.—The effect of pH on the reaction velocity at 37° C. The incubation mixture, containing 3.0 ml. saliva, 2.5 ml. buffer, and 2.5 ml. substrate solution, was incubated for 2 hours. Final substrate concentration was 1.66×10^{-4} M. Measurements (○) and (●) were obtained from a single saliva pool, but tested on successive days

Fig. 2.—Effect of substrate concentration on the reaction velocity. (○) Mean reaction velocity vs. substrate concentration; (●) S/v vs. S.; pH = 8.32.

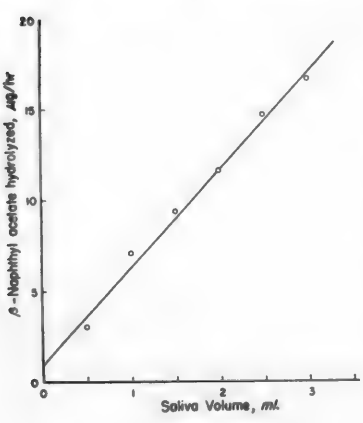

Fig. 3.

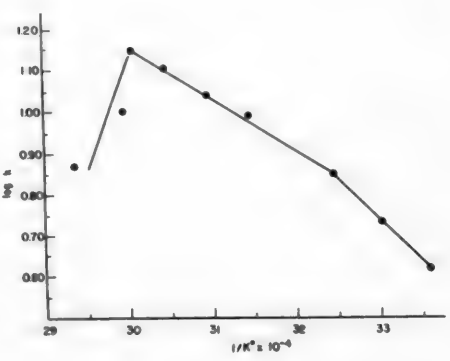

Fig. 4.

Fig. 3.—Effect of enzyme concentration on the reaction velocity. Reaction velocity against saliva volume at pH 8.31 and 37° C. Final substrate concentration of 3.33×10^{-4}M.

Fig. 4.—Effect of temperature on parotid total esterases. Log of the reaction velocity vs. the reciprocal of the absolute temperature.

RESULTS

pH Activity Curve.—Esterase activity was measured over the pH interval 7.0 to 8.8 (Fig. 1). A sharply defined optimum was observed at pH 8.6. However, a large amount of spontaneous hydrolysis occurred at this pH, causing a decrease in the sensitivity of the measurement. Consequently a pH of 8.3 was used for all subsequent analyses.

Enzyme Substrate.—The initial reaction velocities of the 5 substrate concentrations (0.83, 1.66, 2.50, 3.33, and 4.15×10^{-4}M) were plotted against the substrate concentration (Fig. 2). Greater concentrations could not be tested because of the high degree of spontaneous hydrolysis encountered when solutions above 4.15×10^{-4}M were incubated for more than 2 hours. A Lineweaver-Burk plot of S against S/v produced a straight line whose slope was equal to 1/V, and ordinate intercept equal to Km/V. The maximum velocity, V, and Michaelis-Menten constant, Km, were calculated to be 47.6 μg per hour and 1.45×10^{-3}M, respectively.

A linear relationship was observed between the saliva volume (enzyme concentration) and the amount of substrate hydrolyzed (Fig. 3).

Temperature and Enzyme Activity.—The activation energy of the salivary total esterase activity was determined using the Arrhenious equation $\log K = \frac{-\mu}{2.303R}\left(\frac{1}{T} + C\right)$. Assays were carried out at 298°, 303°, 308°, 318°, 323°, 328°, 333°, 334°, and 341° K. employing a 1.66×10^{-4} substrate concentration and a pH of 8.33 (Fig. 4).

The activation energy was calculated as 5,900 cal. per mole. A possible transition point similar to that noted with lipase,[7] jackbean urease,[7] and salivary acid phosphatase,[8] was observed at 35° C. Maximum activity occurred at 60° C. The temperature coefficient (Q_{10}) for the interval 35° to 45° C. was 1.38.

DISCUSSION

Beta-naphthylacetate is attacked by nonspecific esterases, cholinesterases, and lipases. Thus, it may be expected that these enzymes, known to be present in parotid saliva, contribute to its hydrolysis. The nonspecificity of the substrate for these different enzymes is evidenced by the data which shows that the whole group apparently can be described as a single enzymic activity. The substrate affinities of the group were several times less than those found for human serum esterases in which the values for Km were observed equal to 4.15×10^{-4}M with p-nitrophenylacetate and 2.5×10^{-4}M with p-nitrophenylpropionate.[9] However, optimal pH values of 8.4 to 8.6 agree very well with those reported. Glick[10] has also recorded an optimum pH of 8.4 to 8.5 in the splitting of acetylcholine by serum. Although certain similarities in the properties of the serum esterase and parotid saliva total esterase activities are evident, certain dissimilarities also occur. In addition, the lack of prior investigation of the kinetic characteristics of mammalian esterases employing β-naphthylacetate as substrate affords no real basis for comparison of the salivary enzyme. The identity of the salivary total esterases can possibly be resolved by studies on the hydrolysis kinetics of β-naphthylacetate by other esterases present in the human body.

SUMMARY

Investigation of the enzymatic hydrolysis of β-naphthylacetate by human parotid saliva indicated a pH optimum of 8.6 for parotid saliva total esterases.

The Michaelis-Menten constant was calculated to be 1.45×10^{-3}M. A linear relationship between saliva volume and amount of substrate hydrolyzed was observed. The energy of activation was 5,900 cal. per mole. Maximum activity occurred at 60° C. The temperature coefficient (Q_{10}) for the interval 35° to 45° C. was 1.38

The authors would like to express their indebtedness to Mrs. Vilma M. Johnson for her technical assistance.

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IS COLLAGENASE FOUND IN SALIVA FROM PERIODONTAL DISEASED MOUTHS?

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IT HAS been stated[1] that the enzyme, collagenase, theoretically could have some relationship to periodontal disease.

Enzymes in the oral cavity may originate from several sources: the major and minor salivary glands, bacteria, oral tissues, and ingested substances. It is of primary importance, therefore, to have some fundamental knowledge concerning the various sources of the enzymes present. An attempt toward achieving this end is presented.

MATERIALS

The enzyme was harvested from cultural filtrates of neopeptone, proteus peptone, and a beef infusion media. Each was inoculated with a strain of *Clostridium histolyticum* and incubated anaerobically at 37° C. for 16 to 24 hours.[2] The culture was filtered through a Seitz filter, and collagenase was isolated by the method described by Oakley, Warrak, and Van Heyningen[3] and Bidwell and Van Heyningen.[4, 5]

The same procedure was followed using rubber band stimulated whole saliva collected from 40 patients with various periodontal disturbances which included: parietal abscess, pericoronitis, chronic Vincent's infection with periodontitis, mild gingivitis, and periodontitis. Saliva samples were processed immediately upon collection; control saliva samples were taken from subjects who were clinically orally healthy.

Azocoll employed here is a reddish purple, insoluble powder prepared in the manner described by Oakley, Warrak and Van Heyningen[3] and supplied by Roth.[1] Rat tail collagen paper was prepared in our laboratory by the method of Delaunay, Guillaumie, and Delaunay.[6]

The gelatin used was a 10 per cent aqueous solution of the Difco (standardized) product.

METHODS

I. *The proteolytic activity on azocoll substrate.*[5] Azocoll is disintegrated when incubated with collagenase; the action of the enzyme is indicated by the liberation from the insoluble azocoll particles of a soluble red dye which can be measured spectrophotometrically. It should be pointed out that azocoll is known also to be susceptible to proteolytic enzymes other than collagenase.[7]

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Three milligram samples of azocoll were incubated at 45° C. with various samples of whole saliva from patients with periodontal disease cases; hyaluronidase (Wyeth, Inc.); pepsin (Armour, crystallized, porcine); trypsin (Armour, recrystallized, bovine); and collagenase. The preparations were dissolved in 5 c.c. of .05 M PO_4 buffer with pH. 7 and 1 drop of 10 per cent phenol added to each tube. All preparations were found free of bacterial contamination at the end of incubation. The preparations were examined at the end of 5, 10, and 30 minutes, and 1, 2, 4, 6, and 24 hours for the liberation of a red dye indicating azocoll breakdown. The enzyme activity was stopped at the desired time by filtering off the undigested particles of azocoll and measured with a Beckman spectrophotometer at 552 mμ.

II. *The action on unaltered collagen*: Five milligrams of collagen were suspended in a collagenase solution, in whole saliva from patients with periodontal disease, and in water. These samples were incubated at 37° C. (pH 7.0 to 7.8) to demonstrate the presence of true collagenase by disintegrating unaltered collagen as a specific substrate over a 24-hour period. Tyrosine determinations were then performed by spectrophotometric procedure to detect the digestive power of the samples. Oakley and others[3-5] have demonstrated that unaltered collagen is a specific substrate for collagenase. If any digestion takes place on unaltered collagen, tyrosine groups are liberated.[5]

III. *The action of the enzyme on gelatin*:[2, 8, 9] A nonspecific method was devised in which the collagenase activity could be estimated from its ability to liquefy gelatin solution under standard conditions. Simultaneous incubation with gelatin (37° C.) was made of tubes containing: (a) "periodontal disease saliva," (b) collagenase, and (c) clinically healthy saliva in a water bath for 1 hour with periodic shaking and then transferred to a bath of ice water. The time taken for the mixtures to set to a consistency such that they failed to flow when the tubes were tilted horizontally was determined and plotted against the dilutions. Collagenase has the ability to digest the gelatin to such an extent that the mixture loses the capacity to form a solid gel on cooling.

In addition, carboxyl groups were determined by titration with alcoholic KOH to follow hydrolysis of gelatin by the saliva.[2] As a further test of the presence of the enzyme, Fe^{++}-SH activators were added to several preparations of saliva in gelatin. Hydrolytic activity was absent both prior to and following the addition of the activators.[8]

IV. *In vivo necrotizing effect.* The ability of saliva from patients with severe periodontal disease to produce hemorrhagic and necrotic lesions in the skin of the rabbit following an intravenous injection of 1 per cent trypsin blue dye was investigated. The exact mode of action of toxic substances in producing skin necrosis has not been fully investigated, but the edematous and hemorrhagic appearance of the lesions suggests an increase in capillary permeability.[10]

RESULTS

In this report no attempt was made to quantitate the collagenase titer of samples in Q units per milliliter[4, 5] since the primary interest was to detect the presence of the enzyme.

I. *The proteolytic activity on azocoll substrate*: Disintegration of azocoll was effected by the enzyme collagenase isolated from bacterial filtrates and trypsin. No evidence of breakdown was observed with "periodontal disease saliva" and hyaluronidase.

II. *Action on unaltered collagen*: Only collagenase isolated from bacterial filtrates was effective in promoting the complete digestion of collagen. There was no disintegration of any saliva samples with collagen over a period of 72 hours' incubation.

III. *The action of the enzyme on gelatin*: The results indicate only collagenase isolated from bacterial filtrates liquefied gelatin. Saliva from patients with periodontal disease had no effect on gelatin; furthermore, Fe^{++}-SH activators had no effect on inducing hydrolysis in the saliva gelatin mixtures.

IV. *In vivo necrotizing effect*: It was shown that only collagenase was active in producing such an effect. Staining developed at the site of injection of collagenase due to the leakage of dye-stained blood fluid into the cutaneous tissues. The effect was produced in less than 24 hours with a dose containing 2 mg. of collagen from cultural filtrate. The lesion also suggested some muscle disintegrating activity with necrosis. Saliva samples did not produce any skin necrosis.

TABLE I
SUMMARY OF ACTIVITIES OF SALIVA OBTAINED FROM PERIODONTAL DISEASED MOUTHS AND CONTROLS

TEST MEDIA AND CLINICAL DIAGNOSIS	TOTAL NUMBER OF CASES TESTED	AZOCOLL BREAKDOWN	ACTION ON UNALTERED COLLAGEN	GELATIN LIQUEFICATION	IN VIVO NECROTIZING EFFECT
Pooled seitzed saliva from periodontal diseased mouths (severe)	30	0	0	0	0
Collagenase from cultural filtrate of Clostridium histolyticum	4	+	+	+	+
Parietal abscess	4	0	0	0	0
Pericoronitis	5	0	0	0	0
Mild gingivitis and periodontitis	3	0	0	0	0
Chronic Vincent's infection with periodontitis	2	0	0	0	0
Clinically normal saliva	5	0	0	0	0

0 = No activity; + = Very active.

DISCUSSION

The tests employed in this laboratory were specific to detect the activity of the enzyme collagenase. The failure to provide evidence that this enzyme could be found in saliva from periodontal conditions suggests the following possibilities: (a) it is not present, or is present in concentrations so small as to exert minimal effect on healthy collagen, or (b) as a product of microorganisms it accumulates in recesses which protect it against ready contact with saliva and obstruct or prevent the effects of ordinary oral hygiene practices.

The possibility was considered that inhibition occurred in the saliva while isolating the enzyme due to neutralization by naturally occurring lytic enzymes which destroyed collagenase. This, however, could not be confirmed when collagenase was added to normal saliva and incubated with gelatin, azocoll, and unaltered collagen, indicating no impairment in proteolytic activity.

SUMMARY

1. Seitzed saliva taken from mouths of patients with periodontal disease does not possess the property of breaking down gelatin, azocoll, or unaltered rat tail collagen.

2. The proteolytic activity of collagenase upon collagen paper is confirmed.

Grateful acknowledgment is made of the cooperation and assistance of Major Julius Godwin, 3510th USAF Hospital, Randolph Air Force Base, Texas, in the collection of various types of periodontal saliva.

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EFFECTS OF INHALATION OF AIRBRASIVE POWDER

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WITH the introduction of the airbrasive technic for preparation of cavities in teeth, it was realized that both patient and operator would be exposed to airbrasive powder, which, according to the manufacturer, was of the following composition: Al_2O_3, 94.56 per cent; SiO_2, 2.23 per cent; Fe O, 0.41 per cent; Ca O, trace.

Numerous reports expressing a wide variation of opinions as to the effect of the inhalation of alumina dust have appeared. Many of the animal experiments reported are inconclusive and the clinical and autopsy findings, although suggestive of specific changes, do not present positive agreement as to the toxicity of alumina.

Because inhalation of any dust, especially one containing silica, may cause pneumoconiosis and because of the conflicting reports on the effect of alumina dust and the presence of silica in the airbrasive powder, it seemed desirable to pursue this study.

Industrial dusts are commonly classified into 3 groups based upon tissue reaction to the dust[1-3]: (1) dusts which are absorbed without appreciable tissue alteration or damage; (2) dusts which remain inert within the living tissues, neither being absorbed nor causing proliferative changes; and (3) dusts which irritate and initiate cellular proliferation, fibrosis, and retrograde changes. Groups 1 and 2 are referred to as harmless dusts and Group 3 as harmful dusts, but all types of dusts may interfere with normal respiration and cause bronchial irritation or obstruction if they are inhaled in large enough quantities. The present knowledge of the biologic effects of aluminum-oxide dust is based upon animal experimentation, clinical findings in human beings, and a few autopsy reports.

Since Arnold[4] introduced the technic of exposing animals to dust in dust chambers, this method has been widely used for investigation of the biologic effects of various types of dust. Most of these investigations have been concerned with the effect of various types and mixtures of silica and other related industrial dusts, but few experiments have been reported on aluminum-oxide and hydroxide.

Haynes[5] exposed guinea pigs in dust chambers to Al_2O_3. His animals demonstrated pulmonary fibrosis after 9 weeks of exposure. The fibrosis was

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progressively more severe in those having longer exposure. The fibrosis decreased in the animals after exposure was terminated and was minimal in those sacrificed after a long period. Denny, Robson, Irwin[6] exposed rabbits to aluminum powder for 14 months. The lungs contained 270 to 1,200 mg. of powder, but the animals showed minimal clinical and histologic change. Jötten and Eickhoff[8] reported the most extensive dusting experiment using aluminum-oxide. Dusting was heavy and most of the animals died of dust exposure. Marked pulmonary changes were produced, but the findings were not consistent for all animals. The observed changes were thought to be due to "aluminosis." The changes were similar to those described in human beings by Kahlan.[23] Injection studies with $Al_2 O_3$, reported by Gardner and Cummings[10] showed the aluminum-oxide was phagocytized and incapable of producing fibrosis after 2 to 3 years.

Human beings have been studied by Sutherland, Meiklejohn and Price,[12] Doese,[13] Goralewski,[14-18] Shaver and Riddell,[19] and Shaver.[20] Sutherland, Meiklejohn, and Price and Doese could find no specific changes produced by alumina, but Goralewski, Riddell, and Shaver demonstrated definite clinical and roentgenologic findings. Autopsy findings attributed to alumina were described by Kireh[22] and Kahlan.[23]

The mechanism of irritation in the various types of pneumoconiosis is not well established. The chronicity and the nonspecificity of the pneumoconioses and the individual variation in response from one species to another, and from one organ to another, variation to response to the particle size, to chemical and physical characteristics of the material, to type of exposure, to length of exposure, are all important factors in the study of this group of diseases. A well-controlled experimental study or clinical observation requires a consideration of all these factors which makes such investigations very difficult. Furthermore, an interpretation of the materials which are influenced by so many variable factors will tend to be subjective, especially with reference to clinical observation, and to the evaluation and selection of limited pulmonary fields suitable to prove or disprove the theories of the observer. The reported experimental investigations with $Al_2 O_3$ are of limited significance, mainly due to unsatisfactory correlation of the numerous mentioned variants. Aluminum-oxide with particle size larger than 1 to 3 microns seems to be relatively inert in guinea pigs and rabbits. Pulmonary lesions of aluminosis were experimentally produced by Jötten and Eickhoff in rabbits, but one cannot be sure that the dust which they utilized was pure aluminum-oxide, since it was factory dust, which had not been analyzed. The same objection may be made to the autopsy cases of aluminosis where the individuals had been exposed to factory dust, and the autopsy cases which showed a relatively high percentage of silica present, so no conclusion can be made as to the specific effect of the aluminum-oxide. One very important factor is present in all of the reported cases of aluminosis—the small particle size. The cases of aluminosis in German factories developed after the particle size of the dust was reduced 10 times and the particle size of the fumes in the bauxite factory was also extremely small.

It was found by van Wijk and Patterson[30] that about 25 per cent of particle size 0.2 micron is eliminated by breathing. About 80 per cent of particle size 2 micron is eliminated and at particle size of 5 micron, 95 per cent of the particles are eliminated. Hamlin[31] also stated that very few particles larger than 10 micron will reach the alveoli of the lung.

The literature indicates that the presence of aluminum will not give complete protection against silicosis nor against the silica particles in the dust, so any type of dust where contamination with silica is present should be considered potentially harmful.

METHODS

The methods used to investigate the effect of inhalation of airbrasive powder were as follows:

1. Intraperitoneal injection of 1 Gm. of sterile airbrasive powder in 2 rabbits. (All powder used was of the type used for the preparation of cavities. None of the powder was of the type used for prophylaxis.) Animals were sacrificed at 120 days.

2. One-half gram of sterile airbrasive powder was blown into the trachae of 2 rabbits by mouth pressure. The animals were sacrificed after 207 days.

3. Methods 1 and 2 were found to be unsatisfactory and were discontinued in favor of the following technic: Animals were placed in 112 L. (approximately 30 gal.) barrels having a tight fitting cover which had ⅜ inch holes in either side of the top. A ⅜ inch rubber hose protruded just through the top on one side. This was connected to a 2 L. suction flask which contained airbrasive powder. The top of the flask was fitted with a cork carrying a single glass tube 3/16 inch in diameter. This tube reached nearly to the bottom of the flask and was attached by a rubber tube to the compressed air line. The air pressure was adjusted to provide enough force to blow the powder into the barrel in a constant fine stream. The powder on entering the barrel was dispersed throughout the area as a uniform haze which easily permitted observation of the animals in the bottom of the barrel. Through the other opening in the top, a ⅜ inch rubber tube was passed to the bottom of the barrel. This was attached to the compressed air line having sufficient pressure to make the end of the hose describe a small circle. This re-agitated the dust and also provided an adequate supply of air for the animals, without which they would suffocate. Because of the development of static electricity, the barrels must be grounded (Fig. 1).

This method, called dusting, provided an atmosphere with an intense continuous supply of airbrasive powder. Rabbits, guinea pigs, and monkeys were dusted by this method as follows: 16 rabbits were exposed to new airbrasive powder 6 hours per day for intervals ranging from 13 to 22 days. The average exposure time was 200 days. Some animals were sacrificed immediately following dusting, while others were maintained without further exposure up to 500 days (Table I).

Twenty-five guinea pigs were dusted as follows: (a) Six guinea pigs were exposed to 6 hours per day for 15 days with new airbrasive powder. All animals died within 22 days of the initial exposure. (b) Twelve guinea pigs were exposed to new airbrasive powder 1 hour per day for periods ranging from 18 to

TABLE I
GROUP III, RABBITS (6 HOURS EXPOSURE PER DAY)

ANIMAL NO.	DAYS OF EXPOSURE	TOTAL HOURS	DAYS FROM FIRST TO LAST EXPOSURE	DAYS SINCE LAST EXPOSURE	TOTAL ELAPSED TIME
1	13	78	15	0	15
2	33	198	51	0	51
3	33	198	51	0	51
4	222	1,332	305	0	305
5	219	1,314	293	0	293
6	219	1,314	293	0	293
7	219	1,314	293	0	293
9	115	690	155	0	155
10	115	690	155	0	155
11	115	690	155	0	155
12	115	690	155	0	155
13	198	1,188	300	39	339
14	198	1,188	300	348	648
15	198	1,188	300	154	454
16	198	1,188	300	51	351
17	185	1,188	300	165	465

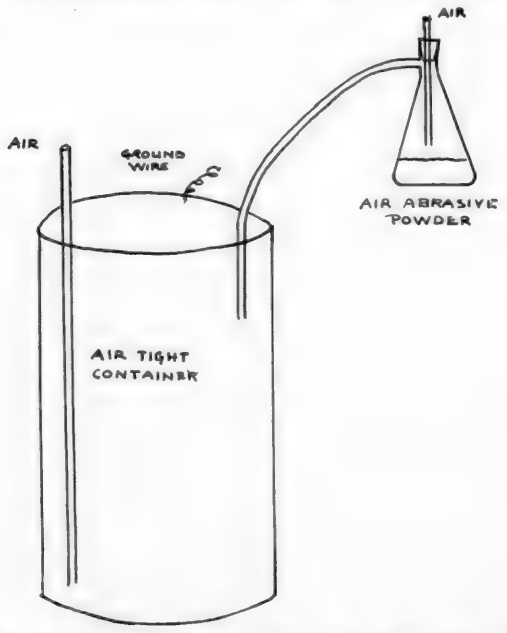

Fig. 1.—Airtight 30 gal. paper barrel (with metal top) which was used as dusting chamber. Air is passed through the block to supply fresh powder. It is also directed through a second inlet to the bottom of the barrel to provide air for animals and to reagitate powder.

205 days. One animal was alive at the end of 475 days. The remainder died at intervals ranging from 22 days to 352 days. (c) Seven guinea pigs were exposed to used airbrasive powder 1 hour per day for 33 days. All animals died in a period of 77 to 85 days (Table II).

Three Rhesus monkeys were exposed in a dust chamber as follows: (a) One was exposed 1 hour per day for 60 days to used airbrasive powder. Time elapsed before sacrifice 94 days. (b) One was exposed 2 hours per day for 60 days to

TABLE II
GROUP IV, GUINEA PIGS

ANIMAL NO.	DAYS OF EXPOSURE	TYPE POWDER	DAYS FROM FIRST TO LAST EXPOSURE	DAYS SINCE LAST EXPOSURE	TOTAL HOURS	TOTAL ELAPSED TIME	
1	15	New	17	0	90	17	6 Hrs. Exp. Per Day
2	14	New	16	0	84	16	
3	15	New	17	3	90	20	
4	15	New	17	3	90	20	
5	15	New	17	5	90	22	
6	15	New	17	3	90	20	
7	18	New	20	0	18	20	1 Hr. Exp. Per Day
8	23	New	25	5	23	30	
9	25	New	33	0	25	33	
10	19	New	21	0	19	21	
11	20	New	22	0	20	22	
12	27	New	35	0	27	35	
13	27	New	35	0	27	35	
14	205	New	275	77	205	352	
15	205	New	275	35	205	310	
16	195	New	275	1	195	258	
17	205	New	275	35	205	310	
18	205	New	275	348	205	623	
19A	33	Used	44	36	33	80	
19B	33	Used	44	41	33	85	
20	33	Used	44	36	33	80	
21	33	Used	44	41	33	85	
22	33	Used	44	33	33	77	
23	33	Used	44	33	33	77	
25	33	Used	44	35	33	79	

used airbrasive powder. Time elapsed before sacrifice 186 days. (c) One was exposed 3 hours per day for 60 days to new airbrasive powder. This animal died at 261 days (Table III).

TABLE III
GROUP V, MONKEYS

ANIMAL NO.	DAYS OF EXPOSURE	TYPE POWDER	HOURS OF EXPOSURE	DAYS FROM FIRST TO LAST EXPOSURE	DAYS SINCE LAST EXPOSURE	TOTAL HOURS	TOTAL ELAPSED TIME
3801	60	Used	1	93	93	60	186
3805	60	Used	2	93	1	120	94
3804	60	New	3	93	168	180	261

At the time of death or sacrifice all animals were autopsied and examined and tissues selected from trachae, lung, heart, liver, kidney, spleen, adrenals, and intestine for microscopic examination. The lungs were cut in cross section from apex to base by means of 6 cuts. Tissue was also selected from any other than the above-mentioned areas if it appeared abnormal. All tissues were prepared by paraffin technic and stained with hematoxylin and eosin.

OBSERVATIONS

Animals, especially guinea pigs, exposed to dust for 6 hours per day developed severe conjunctivitis, hemoptysis, and weight loss. Those exposed for shorter periods appeared to tolerate exposure well. No symptoms were evident and the animals grew and gained weight equal to the control animals.

A summary of the findings for each group of animals follows:

Group I.—Rabbits having intraperitoneal injection of airbrasive powder: At necropsy numerous smooth gray plaques of variable size were present on the surface of the gut and the abdominal wall at the site of injection. These were slender peritoneal adhesions. Microscopic examination of these plaques revealed large quantities of airbrasive powder having an intense foreign body reaction

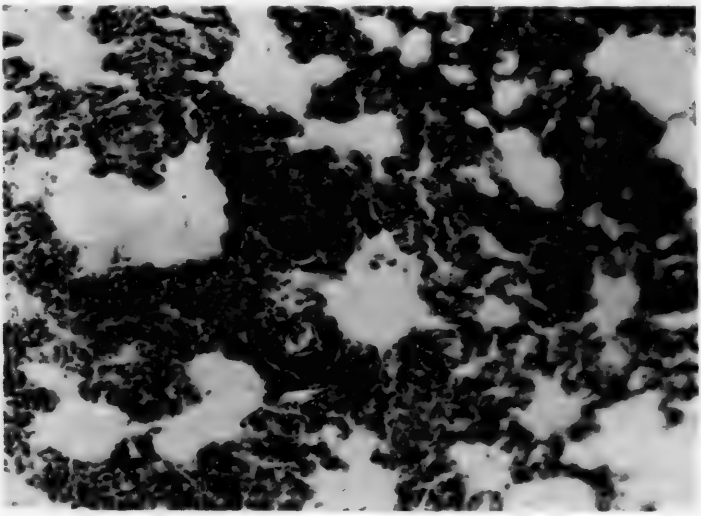

Fig. 2.—Monkey 3805. Focal distribution of airbrasive powder. Thickening of alveolar septa. (H & E stain; ×238.)

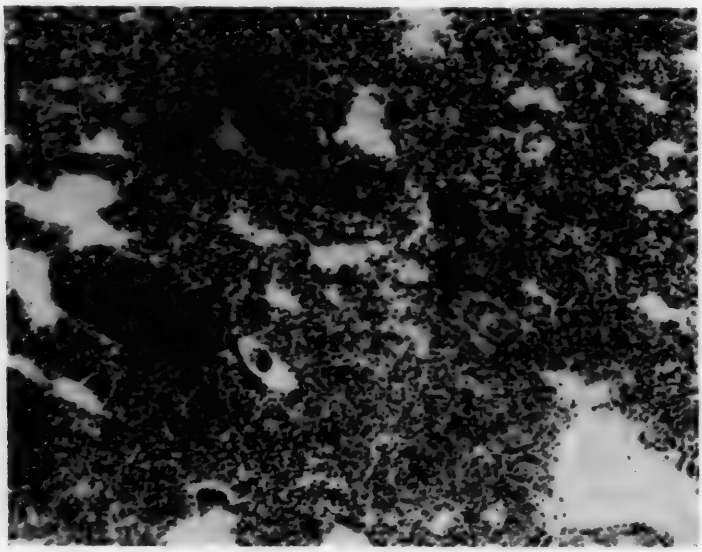

Fig. 3.—Monkey 3805. Consolidation of lung. Active pneumonitis. Focal deposition of airbrasive material. (H & E stain; ×238.)

about the powder particles. Some of the particles were dissociated and appeared as fine black granules. There was minimal evidence of fibrosis and no evidence of necrosis in the surrounding area. The exudative response was minimal.

Group II.—Airbrasive powder blown into trachae: At necropsy the gross changes were minimal except for moderate congestion. When the lungs were

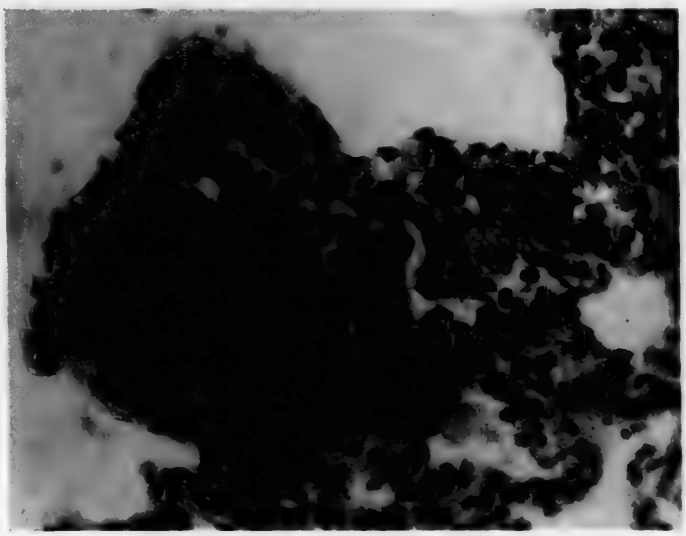

Fig. 4.—Monkey 3805. Perivascular deposition of airbrasive powder. Phagocytized particles and crystals in alveolar septa. (H & E stain; ×840.)

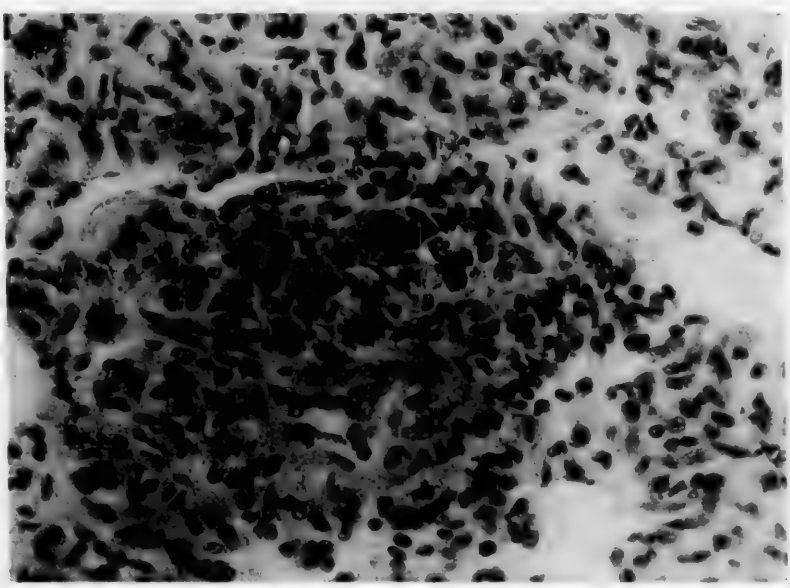

Fig. 5.—Monkey 3805. Airbrasive material with associated fibrosis. (H & E stain; ×840.)

examined microscopically only a small quantity of airbrasive powder was found in the alveolar walls, and this incited only a minimal inflammatory response.

Group III.—Animals exposed in dusting chambers 6 hours per day for varying periods of time: Rabbits. Six of the animals died from suffocation due

Fig. 6.—Rabbit 4. Hyperplastic lymphoid follicle containing fine airbrasive powder. (H & E stain; ×238.)

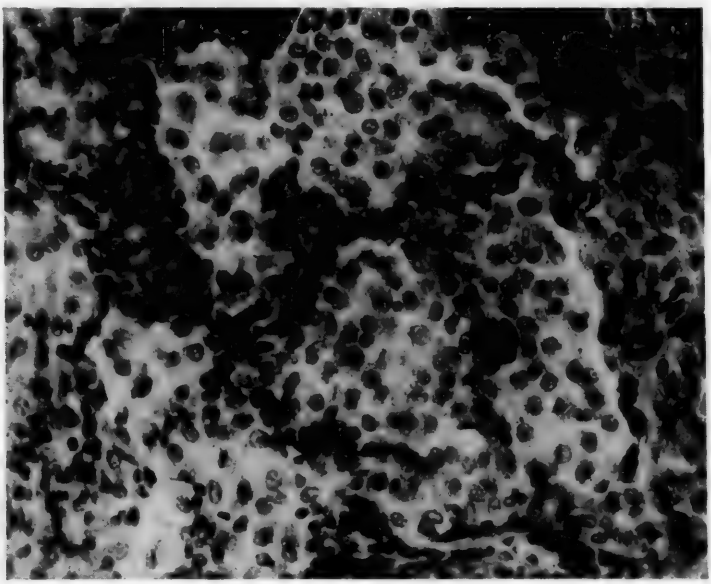

Fig. 7.—Rabbit 4. Granulomatous reaction producing consolidation of lung. Alveoli filled with epitheloid cells. (H & E stain; ×840.)

to the air being shut off, and the others were sacrificed. At necropsy the suffocated animals exhibited only changes of acute oxygen deficiency. The other animals showed acute passive congestion and slight emphysema. Microscopic findings in all animals were intense acute passive congestion, patchy atelectasis and emphysema, pulmonary edema, peribronchial lymphocytic infiltrations with some eosinophils present. Phagocytized dust particles and occasional small crystals of airbrasive material were present (see Fig. 6). The quantity of dust in the lungs was minimal. In the animals exposed for the longer intervals, there was slight fibrosis and slightly more dust present in the lungs.

Group IV.—Guinea pigs exposed in dust chambers: (a) Six animals exposed 6 hrs. per day for 15 days, (b) 7 animals exposed 1 hr. per day for 18 to 27 days, (c) 7 animals exposed 1 hr. per day for 33 days, and (d) 5 animals exposed 1 hr. per day for 205 to 275 days. At necropsy the gross findings were those of congestion, patchy atelectasis, and emphysema. Microscopically, there was evidence of congestion, patchy atelectasis, and emphysema; hyperplasia of rudimentary lymphoid follicles, focal patchy fibrosis, and slight hyaline thickening of basement membrane of bronchioles. There were minimal quantities of phagocytized airbrasive dust. The animals with the 6-hour exposure had a nonpurulent lobular pneumonia and occasional dust crystals in the alveoli. The changes were more severe in the animals receiving the longer exposures.

Group V.—Monkeys exposed 60 days: No significant gross findings were noted at the time of necropsy. Microscopically, there was patchy atelectasis and emphysema; large quantities of phagocytized dust in perivascular and peribronchial areas (Figs. 2, 3, 4, 5); patchy fibrosis (Fig. 3); hyperplasia of peribronchial rudimentary lymphoid follicles (Fig. 6); some crystalline dust in alveoli and peribronchial area and patchy consolidation produced by granulomatous reaction (Fig. 7). Peribronchial lymph nodes contained dust.

STUDIES ON DUST CONCENTRATION IN DENTAL OFFICES

Dust studies were made in 2 dental offices under ordinary working conditions to determine the concentration of dust to which the dentist would be subjected. This study was made by Mr. George Hama of the Bureau of Industrial Hygiene of the city of Detroit.

LOCATION	LIGHT-FIELD COUNT MILLIONS OF PARTICLES PER CUBIC FOOT
1. Dr. D's office, in the breathing zone of dentist using Air Dent.	4
2. (Same location and conditions as sample 1.)	4
3. At discharge of dust collector in Air Dent cabinet. The collector uses an Air-Tec cyclone collector.	13
4. Office of Dr. L, breathing zone of dentist, using Air Dent.	4
5. In the office of Dr. L, general air sample, immediately after using Air Dent.	2

DISCUSSION

All animals showed some reaction to the inhalation of airbrasive material. Those exposed to large quantities developed pulmonary symptoms. A large number of animals died after the dusting period. Rabbits, guinea pigs, and one

monkey all died within a 48-hour period when the temperature was over 100° F. and the humidity high. They died of no apparent reason other than respiratory embarrassment, believed to be due to a reduction in vital capacity. This finding has been observed by other investigators.[8] All animals exhibited some dust in the lung. The amount present was dependent upon the length of exposure time and varied with the type of animal. The rodent type of animals appears to be more resistant to the accumulation of dust than are monkeys. Monkeys with shorter exposure time exhibited a greater quantity of dust in the lungs and a more severe reaction to the dust present. It is apparent from the findings in the lungs of the animals studied that airbrasive powder is toxic when inhaled in large quantities. However, the changes are slow to develop and are not of the severity of those produced by other dusts such as silica.

Because the respiratory system of monkeys is anatomically similar to that of man and because of the apparent natural susceptibility of monkeys to respiratory disease, it appears desirable to use them as experimental animals in studies of this type. More rapid and comparable results could be obtained if monkeys were substituted for the rodent which appears to have a high resistance to pneumoconiosis.

The dust concentration studies in dental offices indicate a low concentration of dust which is below the level considered to be effective in the production of pneumoconiosis, even for dusts containing silica. It therefore appears that the use of the airbrasive technic is not a hazard to the dentist. The airbrasive technic is not used widely and, when used, its application is limited in time. Therefore, exposure is periodic and limited, eliminating the possibility of it being a public hazard even though the dust is toxic.

SUMMARY AND CONCLUSIONS

1. Airbrasive powder can be introduced into the lungs of animals by the dusting technic.

2. The quantity of dust residual in the lung is different for each species and varies within the same species.

3. The rodent type of animals has a high dust tolerance while monkeys have a low dust tolerance.

4. Monkeys are the animals of choice for studies on the effect of dust inhalation.

5. Airbrasive dust in large quantities is capable of producing pulmonary changes which result in reduction of vital capacity.

6. The changes produced are not characterized by fibrosis. They are diffuse rather than nodular and are difficult to produce even with intensive exposure. This would indicate that the material has a low degree of toxicity, even though it can be demonstrated in the lungs in large quantities.

7. Due to the periodic slight exposure obtained by both dentist and patients to a very low concentration of dust, the airbrasive technic does not provide a health hazard.

8. All particles smaller than 3 to 5 microns should be removed from the powder.

9. Although the quantity of silicate is small, it would be desirable to have the airbrasive powder silica free.

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MICROHARDNESS STUDIES OF INTACT SURFACE ENAMEL

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HARDNESS studies of enamel in the past have been carried out almost exclusively on ground sections of teeth.[1-11] These sections were either transverse sections to study the hardness of enamel at various distances from the surface,[4, 9, 11] or smooth polished surfaces prepared within the enamel approximately parallel to the outer surface.[1, 2, 6, 7, 8, 10]

Several types of devices for hardness testing have been used by other investigators, who employed scratch measurements,[2] oscillation speeds of a rocking diamond or ruby cone through enamel,[5] indentation measurements,[5, 7, 8] and other procedures.

Intact surface enamel has a general curvature arising from the shape of the tooth, and local changes of curvature resulting from the perikymata and minute irregularities. Such features become highly important in microhardness tests.

Since the surface of teeth probably is of special significance in the resistance of enamel to the initial carious attack, its hardness and the possible variations are of basic significance. Previous research relating hardness to fluoride treatment,[8] solubility,[10] and abrasion[7] indicates the value of an understanding of the normal hardness of the enamel surface.

APPARATUS AND METHOD

Extracted human teeth which were caries-free and normal appearing were used for the study. The teeth were stored in isotonic saline until the hardness tests, which were made as soon as possible to avoid any possible changes in the surface during storage. Just before the hardness measurements were made the enamel surface was cleaned with water and a rubber prophylaxis cup.

The teeth were mounted in a horizontal position in acrylic resin boxes embedded in hard inlay wax (Peck's Purple) which supported the teeth as rigidly as very hard "stone" (Castone). Drying-out of the surface during the test period did not affect the readings.

The present study was conducted using the Kentron microhardness tester* with a Knoop diamond indenter[12] closely similar to the Tukon hardness tester used by Phillips and Swartz.[8] Impressions were made upon the tooth surface with the diamond indenter by applying a constant load of 500 Gm. for 10 seconds. It is known that for metals the Knoop hardness numbers vary with the test load.[13] Vitovec and Binder[14] reported for polycrystalline materials an

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*Obtained from The Torsion Balance Company, Clifton, N. J.

initial increase of hardness values with an increase in the test loads followed by a slight decrease after a maximum value had been passed. A preliminary study showed that 500 Gm. was heavy enough to give indentations which could be both readily reproduced and give maximum hardness values for enamel surfaces.

The impressions were measured by means of a filar eyepiece in the microscope attached to the instrument. The length of the long diagonal of each diamond-shaped impression was measured in filar units and then converted to microns by the factor of 0.492. From the length in microns, the Knoop hardness number (K.N.) was then derived from a table provided by the manufacturer. The Knoop Number is the load in kilograms divided by the unrecovered projected area in square millimeters.

At least 10 impressions, and usually many more, were placed on each surface under study. Impressions usually were made progressively along a line down the center of the surface from the cervical margin to the incisal edge. Only impressions symmetrical to the eye were measured and recorded. Values are reported for mesial, distal, buccal, labial, and lingual surfaces of teeth.

A perfect indentation has a ratio of the long to the short axis of 7.11 to 1. This ratio could be closely approximated on a calibrated steel block. Slight variations of this ratio for impressions on a steel block appeared to arise from the inability of individual investigators to agree on measurements, especially of the width of impressions, as a result of alterations in focus or lighting.

The corresponding ratios obtained on intact tooth surfaces were found at times to vary significantly from the theoretical value. The most likely interpretation for this variation is that the surfaces are curved. On some teeth, large axial ratios indicated a concave surface and, on some, small ratios indicated a convex surface.

For some teeth, the hardness as calculated from the length of the long diagonal of the impression was corrected to the hardness value corresponding to the actual area of the indentations in square millimeters. This gave a corrected Knoop Number. Usually, however, the symmetry of the impressions was assessed microscopically and only visually perfect impressions were measured.

RESULTS

Hardness of Labial and Buccal Surfaces of Anterior and Posterior Teeth.— The average Knoop Number for 15 anterior teeth was 365 ± 35 (Table I). This value is close to that reported by Souder and Schoonover[7] for tooth sections (350). Although some teeth differ significantly in hardness from the average of the group and from each other, the differences are not great in magnitude. No relation between the age of the patient and the hardness of the enamel was apparent, although the cases studied varied from 16 to 75 years.

The buccal surfaces of 9 posterior teeth had an average Knoop Number of 393 ± 50 as shown in Table II and also showed no effect of age (14 to 44 years). The variation among teeth is similar to that for anterior teeth (Table I). The average hardness is close to the average value recorded in Table I for anterior teeth, and the apparent increase in hardness is not statistically significant at the 0.05 level.

TABLE I
HARDNESS OF LABIAL SURFACES OF ERUPTED HUMAN ANTERIOR TEETH

AGE (YEARS)	TOOTH TYPE*	NUMBER OF READINGS	AVERAGE K.N. ± S.D.	LEVEL OF SIGNIFICANCE†
16	U. canine	9	311 ± 32	.001 < P < .005
16	U. 2nd inc.	23	344 ± 48	.10 < P < .20
35	U. 1st inc.	43	337 ± 58	.05 < P < .10
35	L. 1st inc.	40	417 ± 51	.001 < P < .005
35	L. 2nd inc.	45	385 ± 41	.05 < P < .10
35	L. 2nd inc.	10	406 ± 27	.005 < P < .01
35	L. 1st inc.	10	364 ± 46	.9 < P < 1.
37	L. 1st inc.	12	333 ± 33	.025 < P < .05
37	L. 1st inc.	19	343 ± 23	.025 < P < .05
44	L. 1st inc.	15	399 ± 49	.025 < P < .05
44	L. 1st inc.	18	399 ± 60	.05 < P < .10
46	L. 2nd inc.	44	395 ± 36	.005 < P < .01
46	L. 1st inc.	15	388 ± 63	.20 < P < .25
53	U. 1st inc.	28	353 ± 53	.40 < P < .50
75	U. 1st inc.	22	304 ± 52	.0005 < P < .001
	Average (unweighted) Knoop Number—365 ± 35			

*U. = Upper; L. = Lower.
†Significance in relation to the average K. N. by Student "t" test.[15]

TABLE II
HARDNESS OF BUCCAL SURFACES OF ERUPTED HUMAN POSTERIOR TEETH

AGE (YEARS)	TOOTH TYPE*	NUMBER OF READINGS	AVERAGE K.N. ± S.D.	LEVEL OF SIGNIFICANCE†
14	U. Premolar	13	472 ± 31	.0005 < P < .001
14	U. Premolar	8	343 ± 36	.025 < P < .05
14	U. Premolar	10	431 ± 48	.10 < P < .20
14	L. Premolar	27	417 ± 34	.10 < P < .20
14	L. Premolar	15	462 ± 40	.001 < P < .005
30	L. Molar	14	354 ± 57	.10 < P < .20
33	L. Molar	12	370 ± 46	.30 < P < .40
44	U. Premolar	17	343 ± 26	.001 < P < .005
?	U. Molar	10	345 ± 53	.05 < P < .10
	Average (unweighted) Knoop Number—393 ± 50			

*U. = Upper; L. = Lower.
†Significance in relation to the average K. N. by Student "t" test.[15]

Comparison of the Hardness of Teeth From the Same Mouth.—Four first premolars from a 14-year-old subject were tested on the buccal, lingual, mesial and distal surfaces. The results are shown in Table III. For the most part, all 16 surfaces had similar average hardnesses with the exception of one buccal surface which was apparently softer.

TABLE III
SURFACE HARDNESS OF FOUR FIRST PREMOLARS FROM THE SAME INDIVIDUAL (AGE 14 YEARS)

AVERAGE HARDNESS AS KNOOP NUMBERS ± S. D.				OVER-ALL AVERAGE
BUCCAL	LINGUAL	MESIAL	DISTAL	
343 ± 36	392 ± 38	406 ± 38	367 ± 36	375 ± 37
431 ± 48	441 ± 41	381 ± 29	362 ± 20	404 ± 37
417 ± 33	464 ± 45	465 ± 51	406 ± 61	433 ± 49
462 ± 40	449 ± 66	448 ± 47	408 ± 57	444 ± 52

S. D. = Standard deviation.

These findings were supported by similar measurements of several other teeth. Thus, a molar from a 33-year-old individual had a hardness value of 370 ± 46 on the buccal surface, and of 372 ± 38 on the lingual surface. An upper first incisor from a 75-year-old individual had a hardness value of

304 ± 52 on the labial surface, and 334 ± 29 on the lingual surface. The Knoop Numbers for the buccal and lingual surfaces, respectively, of a maxillary premolar (age 44) were 399 ± 27 and 343 ± 26 and, for a first incisor (age 35), 337 ± 58 on the labial surface and 346 ± 66 on the proximal surface. A lower right first incisor had an average hardness value of 333 ± 33, and the lower left first incisor from the same mouth had a hardness value of 343 ± 23.

Hardness of Primary Teeth.—A limited study of 6 primary (deciduous) teeth disclosed Knoop hardness numbers of 272±26 and 303±16 for a maxillary first incisor and canine, respectively, from an 8-year-old patient. Three maxillary second molars from different individuals had hardness values of 357 ± 56, 388 ± 53, and 399 ± 39, whereas a maxillary first molar had a value of 397 ± 60. With the exception of the somewhat softer canine and first incisor, these primary teeth were within the same hardness range as erupted permanent teeth.

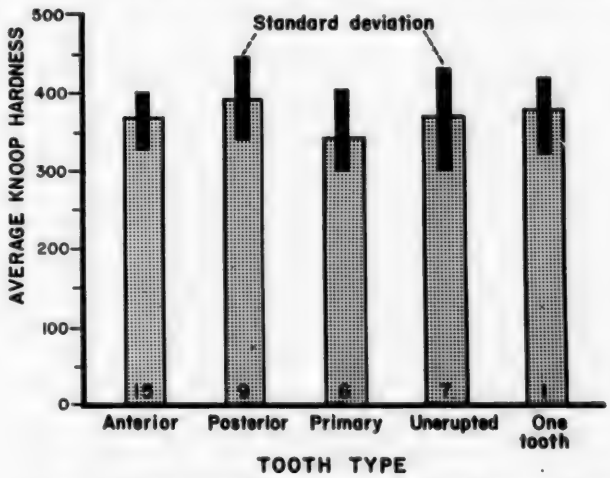

Fig. 1.—Comparison of the hardness of intact enamel surfaces.

Hardness of Unerupted Teeth.—An unerupted maxillary third molar had average Knoop Numbers on the various surfaces as follows: buccal, 440 ± 64; lingual, 386 ± 50; mesial, 413 ± 49; and distal, 385 ± 35. For 3 unerupted mandibular teeth from the same mouth, the Knoop Number for the distal surface of the canine was 322 ± 53, the buccal surface of one premolar was 296 ± 43 and for the lingual of another premolar was 277 ± 16. The third molar was about as hard as the erupted teeth, the canine was about as hard as the "softer" of the erupted teeth, and the premolars were slightly softer.

Variation in Hardness of Areas on the Same Tooth Surface.—The labial surfaces of anterior teeth were studied in detail in order to observe variation of hardness values in areas on the same surface. The hardness values of a lower first incisor ranged from 300 to 463 K. N. on the intact surface, with an average of 399 ± 49. The same surface, ground and polished to eliminate irregularities, showed a range from 308 to 396 K. N., with an average of 348 ± 25.

Table IV gives the hardness values of intact surfaces before and after correction for the variation of the axial ratio of the indentation from the theoretical ratio. A comparison of the standard deviations of these corrected values with those on a polished steel block and on a polished enamel surface indicates that the recorded variation in hardness of intact surfaces is usually the result of local differences in hardness, since the variation in the individual readings is greater than on a polished surface, even after corrections have been made for the effect of the shape of the impression. Moreover, some teeth showed hardness variations of the same order after correction. For example a primary maxillary first incisor, having an average Knoop Number of 272 ± 26, was corrected to 267 ± 25 (Table IV).

TABLE IV
COMPARISON OF KNOOP HARDNESS AND SYMMETRY OF IMPRESSIONS ON DIFFERENT SURFACES

	CALIBRATED STEEL BLOCK	GROUND AND POLISHED ENAMEL SURFACE (67A)	INTACT SURFACE ENAMEL (67A)	INTACT SURFACE ENAMEL (D5)
Knoop Number ± S. D.	527 ± 12	306 ± 14	424 ± 24	272 ± 26
Average ratio of length/width of impressions	7.0	7.8*	6.7†	7.0
Corrected Knoop Number ± S. D.	—	334 ± 12	400 ± 16	267 ± 25

*Slightly concave surface.
†Slightly convex surface.

When the corrected hardness values for the intact surfaces of teeth have standard deviations greater than ± 12, it is concluded that the values represent true local differences in hardness and are not attributable solely to mechanical difficulties in obtaining satisfactory indentations.

The variations observed in hardness on individual surfaces were examined for the possibility of a general trend. Sometimes an area of appreciable size in the cervical region would be harder than in areas in the central or incisal third. Just as often the reverse would be found. Consecutive impressions only 0.06 mm. apart might also differ markedly, but in a fashion not representative of the surface perikymata or other features.

DISCUSSION

A summary of our results is shown in Fig. 1. A comparison of the hardness of different tooth types reveals that all teeth examined were of a similar hardness. The variation in hardness values on different areas on one tooth is as large as that encountered on different teeth.

Different investigators of the microhardness of enamel have reached conflicting conclusions. Many investigators have stated that the intact surface enamel is too irregular to be used for microhardness studies. The present study shows that, with careful selection of suitable indentations, practically all intact surfaces can be tested satisfactorily although with variations somewhat greater than for polished surfaces.

The differences in average hardness values from surface to surface or from tooth to tooth were less marked than anticipated. The extreme limits of Knoop

Number were 272±26 (a first incisor of an 8-year-old subject) and 472±31 (a premolar of a 14-year-old patient). It appeared that, frequently, considerable variations in the hardness can be found in areas on one surface or tooth, but no regular pattern of variation in hardness was apparent on tooth surfaces when different teeth were compared. Marked variation of the hardness on the tooth surface sometimes could be encountered on areas only .06 mm. apart.

The results of the present work do not agree fully with those of Swartz and Phillips[10] on the solubility of enamel at areas of known hardness. These authors stated, "... hardness values remained quite constant on an area 2 mm. in diameter ... the deviation from the average was within acceptable limits." Earlier,[8] the same authors stated that the hardness of enamel could be increased by 5.1 or 7.1 per cent by applying sodium or stannous fluoride, respectively, to tooth surfaces. These findings on polished surfaces of enamel would not appear to be significant in terms of the variations in hardness noted in the present work for intact and ground surfaces. Great variations in hardness were observed within areas 2.0 mm. in diameter, and differences in average hardness of 5 to 7 per cent would seem to be within the experimental error.

Karlström,[3] and Saunsbury and Atkinson[11] have reported that teeth from the deciduous dentition are of different hardness from that of permanent teeth. These workers disagree as to which type of tooth is harder. It appears from the present, somewhat limited, study of deciduous teeth that their hardness is similar to that of permanent teeth.

Contrary to the conclusions of Saunsbury and Atkinson,[11] the age of the tooth seemed to bear no correlation to its hardness. It must be noted, however, that the findings of the present work are mainly for intact surface enamel, whereas all previous work mentioned has been done on ground surfaces or sections.

TABLE V
A COMPARISON OF THE KNOOP HARDNESS NUMBER OF EXTERNAL TOOTH ENAMEL WITH THAT OF SOME METALS*

SUBSTANCE	KNOOP NUMBER
Human teeth (external surface)	380
Range	(250-500)
Hardened steel	732
Hard stainless steel	370
Intermediate steel	251
Soft steel	164
Brass	164
Silver alloys	105
Aluminum alloys	87

*Calculated by Dr. Robert Kattus from data in *Metals Handbook*, American Society for Metals, 1948.

The presence of a discrete harder outer layer of enamel has been reported by other workers, but it seems unlikely that a good approximation of hardness values could be made near the edge of a cut or ground section. A depth of 0.1 mm. was the location of the outermost indentation possible in previous work.[11] By the standards of the current work these measurements were well into the bulk of enamel since the indentations of the current work are less than 5 microns (0.005 mm.) into the

enamel surface. The data on intact and polished surfaces show that intact surface enamel is harder than underlying enamel. The relation of the surface hardness to that of the underlying enamel was not studied further in the present work.

The hardness of the teeth studied fell in the range 250 to 500 Knoop Numbers with an average hardness around 380. This value is similar to the hardness of that of a hard stainless steel (370 Knoop units). Table V records the Knoop Number of several metals, as calculated by Dr. Robert Kattus of the Southern Research Institute.[1]

SUMMARY

With the Knoop indenter, hardness measurements can be made on the intact enamel surfaces of extracted teeth in the outer 0.005 mm. The deviations of hardness values on the intact tooth surface usually are somewhat greater than for polished surfaces. A portion of the deviation seems to arise from the curvature of the surfaces.

The average hardness of the teeth studied was about 380 Knoop Numbers, similar to that for a hard stainless steel (370 K.N). Extreme variations fell in the range 250 to 500 K.N. Variation, almost to these extremes, could be found on some single surfaces, and appeared to represent local variations, with no consistent pattern.

Variations among tooth types in the same and different mouths, between surfaces on the same tooth, between deciduous, erupted, and unerupted teeth, and between teeth from individuals of different ages were not pronounced. The observed variation seemed generally to fall in the range that could occur on a single surface.

The assistance of the following is gratefully acknowledged: Dr. Thomas Jones, Dr. Robert Kattus (Southern Research Institute), James Pigman, and Philip Timberlake.

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AN ABRASION METHOD FOR DETERMINING THE WEAR RESISTANCE OF TEETH

I. Description of Apparatus and Variables

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HODGE tested a number of the most widely used adaptable devices for determining hardness of teeth.[1] Enamel and dentin of human teeth were used in these and subsequent experiments[2-5] concerned with the relative hardness of various tooth structures, sound and carious teeth, and certain metals and minerals. Of the methods tried, the microcharacter hardness tester seemed to be the most suitable for determining the relative hardnesses of various portions of human teeth.

The variable and frequently small size of teeth limits the usefulness of the method in the study of experimentally induced structural changes in teeth. Because of some of these difficulties a new approach was made to the problem.

The development of an abrasion procedure for determining the wearing qualities and relative hardnesses of the teeth of rats is herewith described.

APPARATUS AND PROCEDURE

The apparatus is shown in Fig. 1. The motor (*A*) is connected to the clutch (*B*) which in turn is joined with the drive drum (*I*). The grinding belt* (*G*) moves in the direction indicated by the arrow. The clutch is regulated to give the desired rate of belt speed. An aluminum form that holds the tooth is placed in the vise (*D*) with the back of the form held flush against the plate of the vise to ensure that the tooth contacts the grinding plate at the same angle for each successive grinding. The tooth is cleaned of all extraneous material and stored in 95 per cent ethanol until it is set. A dental cement (Coecal, Coe Co., Chicago, Ill.) is mixed with water to make a paste. The paste is packed into a hole drilled in the aluminum form. The base of the tooth is placed in the cement and more cement is packed around the tooth with a small spatula. If the tooth is a curved incisor, it is placed in the cement so that a tangent to the mid-point of the arc formed by the tooth is perpendicular to the belt during most of the grinding operation. The cement is allowed to harden slightly and then the excess cement is scraped off. After 2 hours, the tooth is stored overnight in 95 per cent ethanol before use.

The feeler gauge (*F*), which is scaled to 1/1000 inch, is fixed to the end of the sample arm (*C*) and its feeler rides against the stop (*N*). The difference

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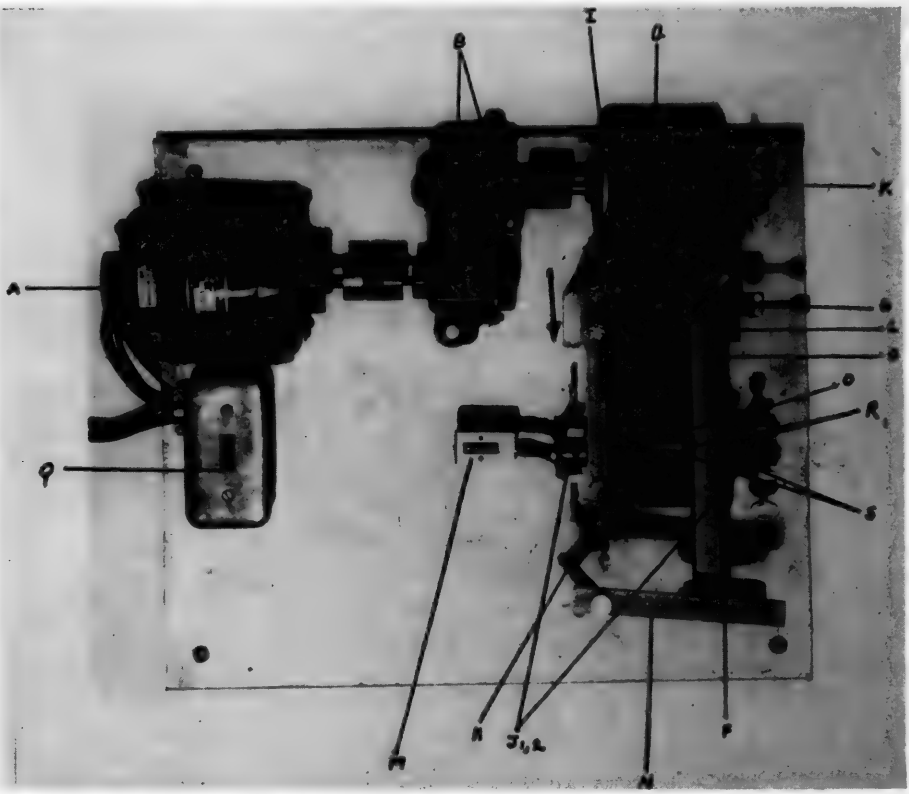
*600 grit, 3x24 silicon carbide, Wetordry Tri-M-Ite cloth belt, Minnesota Mining & Mfg. Co., St. Paul, Minn.

between the readings on the feeler gauge dial before grinding and after grinding measures the wear sustained by the test object. The counter (*M*) is used to determine the rate of belt travel and also to predetermine the number of revolutions of the idler that the tooth is to be ground.



Key to Symbols in Fig. 1—A = Motor, B = Clutch, C = Sample arm, D = Vise on sample arm, F = Dial indicator, G = Grinding belt, H = Drum, I = Drive drum, $J_{1,2}$ = Bearings for drum H, K = Bearing for drum I, L = Grinding plate, M = Revolution counter, N = Dial indicator stop, O = Pivot for sample arm, Q = Switch to start motor, R = Bolts that hold bracket washers in place, S = Bracket washers.

The photograph does not show the water supply that is used to wet the belt or the pan that collects the water. A piece of glass tubing is connected to a water supply with a piece of rubber tubing. The open end of the tube is directed on the top of the abrasive belt between the drum (*I*) and the object holder. A small piece of adhesive tape is attached to the end of the tube in such a manner that it spreads the water over the surface of the belt. When a fresh belt is installed, it is first rubbed with a sponge which has been soaked in an aqueous solution of soap. This removes an oil film which otherwise would not allow the water to wet the belt. A shallow pan with a tube leading to a drain is placed under the belt.

An accurate method for determining the area of the surface being ground is an essential part of this testing procedure. A small amount of red dental wax (Kerr's utility wax) is placed on a glass slide, melted on a hot plate and allowed to flow over the surface of the glass slide. The thickness of the wax is such that some light will pass through and the ground surface of the tooth can be forced through it to the surface of the glass slide. The slide is placed on the stage of the microscope and the image of the tooth impression projected and traced from a glass surface mounted 18 to 20 inches above the microscope stage. The glass mounting should be at such a height that the image of the impression of the tooth is large enough for an accurate tracing. Internal cavities such as the pulp cavity and others are likewise traced, measured, and their areas subtracted from the total surface area of the tooth. The surface areas are measured by a planimeter reading from the outline tracings. The planimeter reading is proportional to the surface area covered by the tracing and it, in turn, is proportional to the area of the ground surface of the test object.

Wear (w) as measured on the feeler gauge is a function of the material (h) as related to wear, the force exerted on the abrasive surface (f), the area of material in contact with the abrasive surface (a), the abrasivity of the abrasive surface (z), the distance that the material moves relative to a point on the abrasive surface (s), and the velocity of movement of the abrasive belt. The effects of these variables have been ascertained from data derived by the use of soft glass rods, Pyrex rods, walrus tusk dentin, or fluorite.

RESULTS AND DISCUSSION

The force (f) is readily held constant and is therefore not of immediate practical importance in the measurements sought. Any differences in weight of the materials intended for use would be a negligible fraction of the total weight impinging on the belt. Results obtained on the investigation of variability in abrasivity (a) over the surface of the belt, and on different belts by using soft glass rods of different sizes but uniform surface areas, have indicated that the belts are of uniform abrasivity. However, in order to ensure strict control, it may be desirable to test the abrasivity of each belt with a uniform reference standard. Soft glass rods have been satisfactorily used for this purpose. Any variation in abrasivity from one belt to another can also be taken into account by running determinations on samples from each experimental group on each belt. Such a procedure has been applied to the determinations on rat teeth described in an accompanying paper.

The relationship between wear and distance of travel (s) is obvious. The change in abrasivity due to repeated use of a single strip of belt was investigated with soft glass rods, Pyrex rods, and walrus tusk dentin. The abrasivity of the belt (defined as $\frac{\Delta w}{\Delta s}$) is a continuously changing variable. Fig. 2 shows that this relationship is described by the power function

$$w = k_1 s^n \qquad (1)$$

in the range tested. Since a family of lines with equal slopes (n = 0.80) was obtained for the different materials tested, the abrasivity, or the rate of change of wear, is independent of these materials and dependent only upon the distance of travel. If the reference standard has the same slope and same surface area as the materials under test, a direct relationship is obtained from comparing wear at any given distance of travel on a single track of belt. Differentiation and integration between the limits s_1, s_2, and w_1, w_2 of equation (1) yields the expression

$$\log \frac{W_2}{W_1} = n \log \frac{S_2}{S_1} \qquad (2)$$

which allows the calculation of wear at any distance of travel. In usual practice of measuring relative rates of wear, the distance of travel is readily controlled.

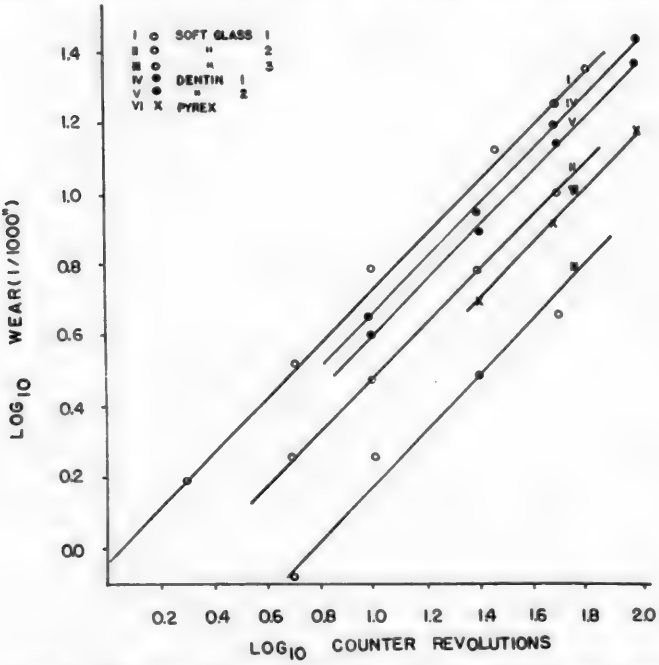


Fig. 2.—The effect of repeated use of the same strip of belt on its abrasivity.

Under this condition the materials are ground under nearly identical abrasive conditions, although the abrasivity of the strip is continuously changing.

Wear per unit revolution of the belt decreases slightly as the rate is increased. This variable is also readily controlled, and a rate of 50 r.p.m. of the counter (20 r.p.m. of the belt) has been found satisfactory.

The data on the relationship between wear and the surface area of the material impinging on the abrasive surface (a) fits the power function expressed by the equation

$$w = k_2 a^{-m} \qquad (3)$$

as seen in Fig. 3. A family of curves with slopes m = –0.75 were obtained for the various materials indicated. Since area is the least controllable variable, formula (3) is applied routinely. Relative rates of wear for a given distance of travel can be obtained by extrapolation at identical areas on the logarithmic plots, or by calculation using the normal equations of the regression line. If two materials of different areas had been ground for different distances, and a comparison of relative wear is desired, the expression (2) and the integrated expression of (3)

$$\log \frac{w_2}{w_1} = -m \log \frac{a_2}{a_1}$$

can be used.

Defining hardness (h) as the resistance to wear, and expressed as

$$h = \frac{1}{w}$$

relative values of hardness can be assigned.

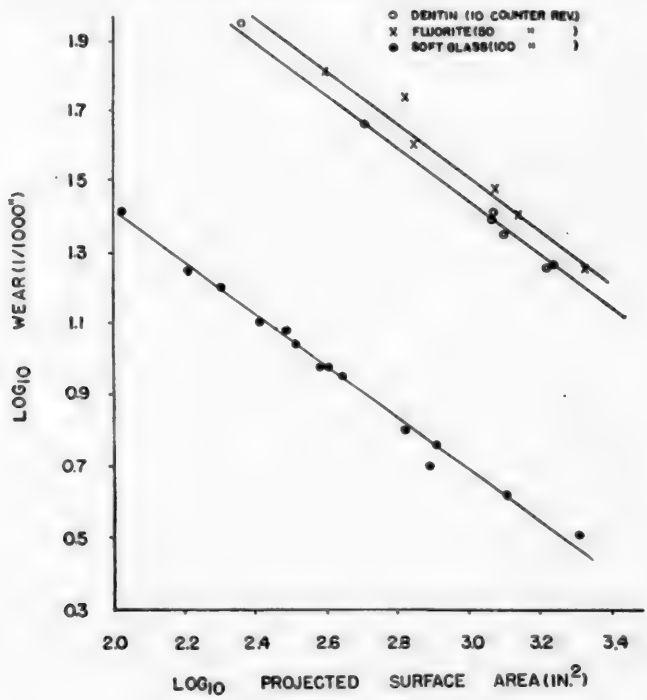


Fig. 3.—The effect of surface area on wear.

Since the area of a rat incisor is not constant throughout its length, several area measurements are taken at different points through the course of its abrasion test. Two methods of expression of data have been tested. Both methods have proved satisfactory. In the first method, which makes use of a single track of belt, the cumulative wear at several points of the incisor and the average area

at these points are plotted. From the linear plot obtained, relative wear per unit area per unit revolution of the belt can be calculated. In the second method, logarithms of wear obtained on fresh strips of belt and the average area at each point of the incisor measured are plotted. The value at the tip of the incisor is neglected because of the very large change in area and wearing quality. Relative values of wear are then obtained as previously described for other materials. Both methods are manifestations of equations (1) and (3).

SUMMARY

An abrasion apparatus for measuring relative tooth wear has been described.

It was found that wear as measured is a power function of both the area of the material impinging on the surface of the belt, and its distance of travel (revolutions of the belt) relative to the abrasive surface. For the materials tested, the slopes were identical for both relationships.

By measuring wear as a function of surface area of the material in contact with the belt, and holding all other variables constant, values of relative wear for different materials were readily obtained. If the different materials have identical areas, the distance of travel on the abrasive surface necessary to cause a given amount of wear can be measured directly.

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SPECTROPHOTOMETRY OF SALIVA

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SPECTRAL analyses of solutions have long been applied to various body fluids for the purpose of clinical study as well as research.[1] The purpose of this study is to determine the typical spectrum of saliva under average conditions and evaluate it in relation to the known solutes present in saliva.

METHOD

Saliva was collected from young male adults by paraffin stimulation after 5 hours of supervised fasting during which time no liquids or solids were allowed to enter the mouth and smoking was prohibited. Each sample was collected in a refrigerated stoppered container. It was centrifuged for 10 minutes at 1,500 g at room temperature. The supernatant was then poured into a separate tube and stored at 4° C. in a stoppered container, while the centrifuge tube containing the sediment was stoppered and the contents were frozen.

All spectral analyses were carried out with an automatic ratio recording spectrophotometer with a working range from 190 mμ to 2,000 mμ wave length. This report is limited, however, to the ultraviolet and visible region.

RESULTS

Five milliliters of saliva supernatant were diluted 1:3 with distilled water. Three milliliters of this were placed in a silica absorption cell with a light path of .999 cm. and scanned from 200 mμ wave length to 700 mμ wave length. This was repeated for over 200 different samples of saliva from 60 different cases. Examples of the typical absorption spectra curves from the clear and colorless samples of saliva are illustrated in Fig. 1. All samples exhibit a peak of absorption near 280 mμ with slight shifts of ± 3 mμ in position from one sample to another. It will be noted that sample No. 42 shows approximately ½ the absorption of sample No. 36. A hump in the absorption curve was noted occasionally near 290 mμ as in Case 8.

The strong absorption occurring below 230 mμ was eventually resolved into a peak near 200 mμ when the samples were diluted to 1/60 concentration. However, the sensitivity of the spectrophotometer had to be increased to such an extent that quantitative data were difficult to obtain.

The opinions or assertions contained in this article are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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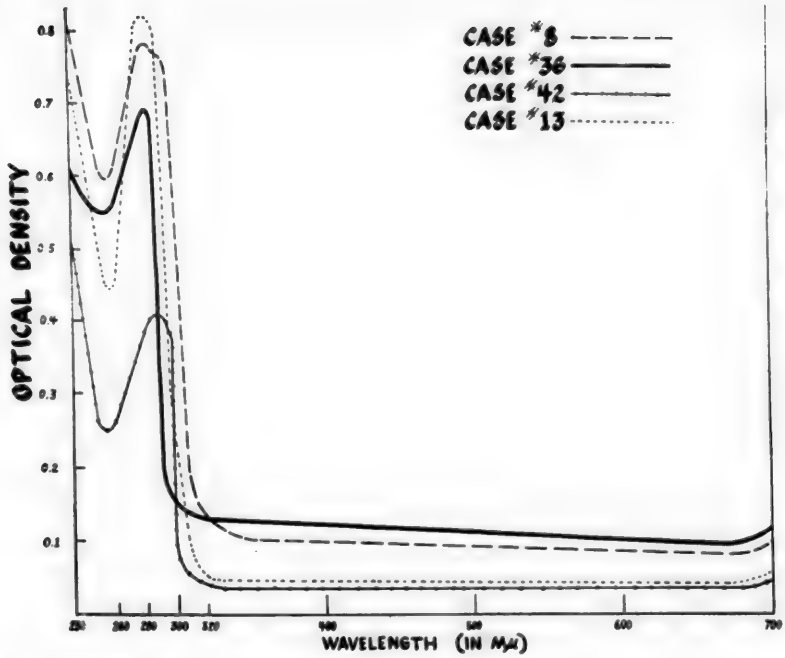

Fig. 1.—Absorption Spectra of Saliva (colorless samples). Protein absorption of centrifuged saliva samples (dilution of saliva: water is 1:3).

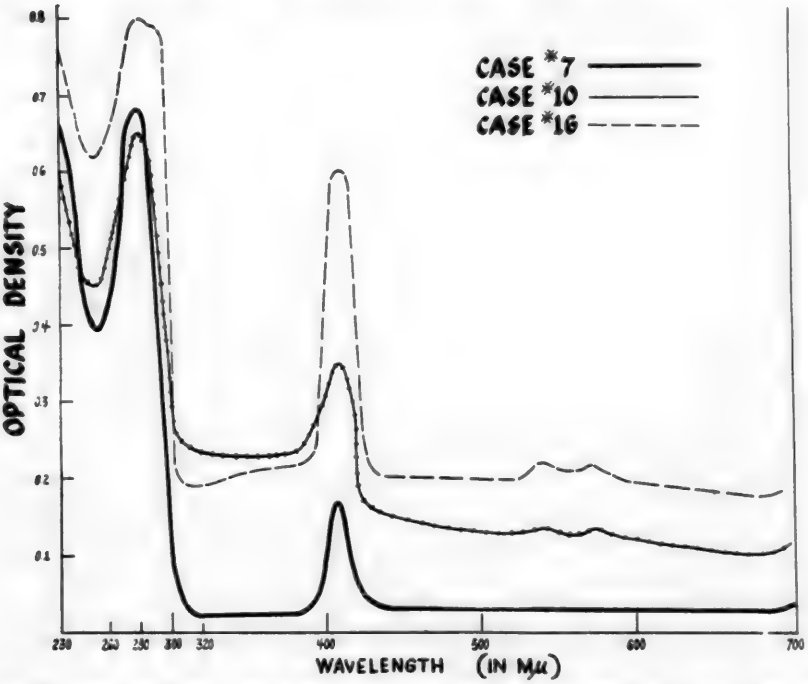

Fig. 2.—Absorption Spectra of Saliva (yellow samples). Protein and blood absorption of centrifuged yellow saliva samples (dilution of saliva: water is 1:3).

The absorption curves of the samples that were yellow in color (representing about 30 per cent of the samples) are illustrated in Fig. 2. Again a sharp peak occurred near 280 mμ but, in addition, a strong absorption peak occurred at 415 mμ with weaker absorption peaks at 540 mμ and 575 mμ. In slightly yellow samples the 415 mμ peak shifted to various positions between 407 mμ to 415 mμ.

Water extracts of frozen-and-thawed saliva sediments contained only one absorption peak at 260 mμ for all samples (Fig. 3). All samples were slowly thawed in order to effect a liberation of the cellular contents of the sediment.

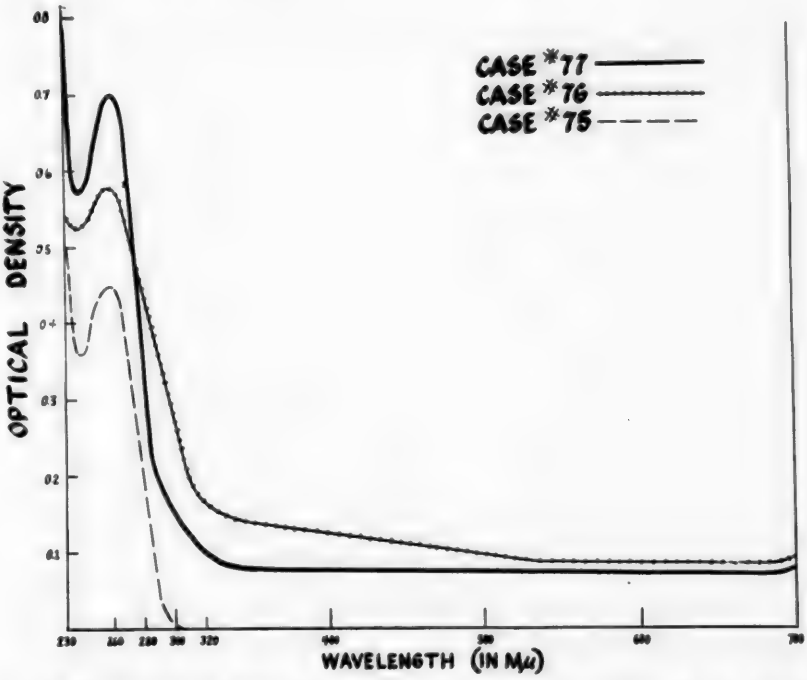

Fig. 3.—Absorption Spectra of Saliva. Nucleic acid absorption of the frozen-thawed extracts of centrifuged saliva sediments (15 ml. of whole saliva were centrifuged, the sediment was resuspended in 10 ml. of distilled water and frozen slowly. Then it was allowed to thaw at room temperature undisturbed. It was centrifuged again and read in the spectrophotometer).

DISCUSSION

Fig. 4 illustrates examples of absorption of saliva spectra. There were no saliva samples that contained absorption peaks at positions other than those shown here.

The absorption peaks occurring near 280 mμ wave length are known to be due to aromatic amino acids and proteins most of which contain aromatic amino acids.[2] The typical absorption spectrum of the saliva of Case 36 (Fig. 1) can be duplicated by using 0.1 per cent purified egg albumin and of Case 42 by 0.05 per cent purified egg albumin.

The hump in the absorption curve at 290 mμ, as illustrated in Case 16 (Fig. 2), was found on further study to be dialyzable against running water at 20° C. The hump could be reconstituted by adding 0.5 mg. of neutral uric acid to 100 ml. of saliva sample.

The strong absorption band below 230 mμ is a characteristic of the amino acid couplings which form the backbone of protein molecules.[3]

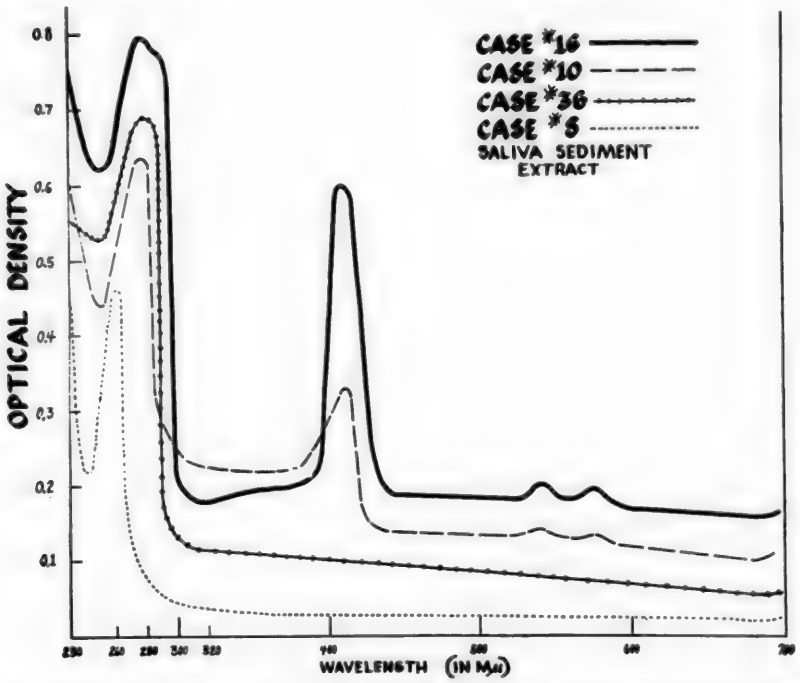

Fig. 4.—Summary of all saliva spectra including samples that were colorless, yellow, and derived from the sediment.

Colored samples exhibited absorption spectra in the visible region which are characteristic of oxyhemoglobin[4] thus suggesting the adulteration of saliva with blood during its collection by the action of chewing wax. A sample of saliva having 0.5 per cent solution of whole blood as an adulterant produced a similar curve to Case 16. The peak for slightly yellow solutions shifted to various positions between 407 mμ and 415 mμ. These peaks were approximated by using various concentrations of hemoglobin (Bacto) in the 0.05 per cent range.

The absorption peak at 260 mμ could be duplicated by a water extract of 0.05 per cent yeast; this value then represented nucleoprotein (nucleic acid–protein) rather than nucleic acid alone.[5, 6]

Light scattering became progressively troublesome as measurements were continued into the ultraviolet, and the level of each curve above the base line was influenced by the amount of light scattering produced in hazy solutions.[7]

Therefore determinations were carried out well into the long wave length side of the spectrum in order to provide a reference line or reference point. A reference point was chosen at 320 mμ and the height of each peak above this point was taken as a measure of absorbant in solution.

CONCLUSIONS

1. In the spectral analyses of 200 saliva supernatants between 230 mμ wave length and 700 mμ wave length the typical absorption curves exhibited peaks at 280 mμ. In addition, many of the samples which were yellow in color exhibited peaks at 415 mμ with minor absorbances at 540 mμ and 575 mμ.

2. The peaks at 280 mμ and 415 mμ were independent of each other and varied from case to case.

3. The spectral analyses of the extract of numerous saliva sediments demonstrated a peak at 260 mμ.

4. The peaks demonstrated by the spectra of saliva were reproduced at 280 mμ by 0.1 per cent protein (albumin) solution, at 260 mμ by 0.05 per cent nucleoprotein (yeast), at 415 mμ by 0.05 per cent hemoglobin (Bacto), and the hump at 290 mμ by 0.5 mg. per cent uric acid.

5. The height of all peaks was duplicated by known concentrations of solutes similar to those present in saliva.

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IRON CONTENT OF TEETH IN HEMOSIDEROSIS

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IN THE COURSE of studies on the chemical basis of metal-mordant stains, determinations of the iron content of many tissue specimens have been performed. The results of analysis of teeth from a girl with hypoplastic anemia and transfusional hemosiderosis are reported.

A 9-month-old white female was first admitted to the hospital because of anemia. Laboratory studies showed hemoglobin 4.3 Gm. per cent and a red blood count of 1.42 million. Bone marrow aspiration showed normal myeloid and lymphoid cells, but severe depression of the erythroid series. The diagnosis of hypoplastic (pure red cell) anemia was made. Over the next 7 years she received treatment at various times with vitamin B_{12}, folic acid, other vitamins, liver extract, cobalt, ACTH, cortisone, and other medications, as well as blood transfusions at roughly 2-month intervals. Except for one transient response to cortisone, the red count, reticulocyte count, and bone marrow erythroid cell count were persistently reduced. During the total period preceding extraction of the teeth analyzed for iron, she received approximately 21,600 c.c. of blood (estimated 11 Gm. of iron). The course of eruption of her teeth was not detailed. On first admission only the upper and lower first incisors were present. Two years later her teeth were noted to be carious and, at the age of 3 years 10 months, 6 deciduous teeth were extracted because of caries. Shortly after this, biopsies of skin and bone marrow showed mild hemosiderosis. Two years later, splenectomy and liver biopsy were performed; the liver showed marked, and the spleen moderate, hemosiderosis. When the patient was 7 years 8 months of age, extraction of the following teeth was performed because of caries: deciduous upper left first molar, second molar and canine, upper right first molar and canine and lower right cuspid; permanent upper right first molar and lower left first molar.

Procedure: Two of the teeth (identity not recorded) were saved for iron determination. The procedure was performed by a modification of the method of Gubler, Lahey, Chase, Cartwright, and Wintrobe.[1] The teeth were crushed, weighed, and digested in concentrated sulfuric and nitric acids (⅔ parts). Perchloric acid, nitric acid, and iron-free water were added, and the solution was heated until colorless. Centrifugation for 10 minutes at 2,000 r.p.m. was used to remove $CaSO_4$ crystals, and thioglycolic acid and orthophenanthroline were added to aliquots of the supernatant fluid. The solution was made alkaline with sodium acetate, and the color intensity determined with a Beckman

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spectrophotometer at a wave length of 510 mμ and a band split of 0.04 mm., using cuvettes with a 1 cm. light path. The results of analysis of the patient's teeth, and of teeth of several normal children of different ages, are shown in Table I. Because of the possibility that some of the iron content of the patient's teeth was due to adherent blood, some of the control teeth were treated by soaking for 24 hours in distilled water (to hemolyze red blood cells), or in normal saline, whereas others were fixed in 10 per cent formalin.

TABLE I
AVERAGE IRON CONTENT OF TEETH, EXPRESSED IN MICROGRAMS PER GRAM OF WET TISSUE (= PARTS PER MILLION)

	NONCARIOUS			CARIOUS		
PRETREATMENT	DIST. WATER	SALINE	FORMALIN	DIST. WATER	SALINE	FORMALIN
Hemosiderosis						50.8 (2)
Controls	5.5 (3)	3.2 (2)	11.6 (2)	8.7 (3)	11.1 (3)	16.1 (6)

The values in parentheses are the number of teeth analyzed in each category.

COMMENT

The data presented demonstrate that the iron content of the teeth may be elevated in transfusional hemosiderosis. Although the authors have been unable to find published data, it can perhaps be presumed that dental iron levels are also elevated in idiopathic iron-storage disease (hemochromatosis), in alimentary hemosiderosis, and in the nontransfusional hemosiderosis associated with certain types of anemia.[2-6] Since the site of the iron deposition is not known (whether pulp, dentin, enamel, or combinations thereof) only speculation as to the mechanism of the increase in dental iron can be offered. It is possible that the iron is deposited in the mineral component of dentin or enamel during the period when these are initially laid down. This concept is supported by the fact that none of the teeth analyzed in this study had yet erupted at the time the patient began her series of blood transfusions. Information on the iron content of teeth of patients with idiopathic hemochromatosis would be of interest in this regard, since this disease never becomes manifest during childhood.[3] An alternative possibility is that the iron in such teeth is deposited from the saliva. Although the iron content of saliva in patients of this type is apparently not known, salivary gland ducts and acini are commonly affected in hemosiderosis,[2, 4] as are also the pancreas, bronchial glands, and sweat glands. The elevated iron content of the sweat of patients with hemochromatosis is well known.[4] It is, therefore, possible that the iron content of the saliva of patients with hemochromatosis is elevated, and that the iron content of their teeth is increased by absorption of salivary iron. Sognnaes[8] and Sognnaes, Shaw, and Bogoroch[7] have demonstrated that I^{131} and P^{32} are absorbed by teeth in detectable quantity from saliva, and Brudevold and Stedman[9] have shown that the lead and tin content of teeth increases with age throughout adult life. These latter authors concluded that tin was absorbed directly by teeth. That metals other than calcium can be deposited in significant quantity in erupted teeth is also suggested by the yellow discoloration of teeth reported to occur in chronic industrial cadmium poisoning.[10] Whether hemosiderosis enhances the susceptibility of teeth to dental caries is apparently not

known, nor whether, if such an effect occurs, it results from chemical alterations of the teeth or of the saliva.

Since the teeth of the patient studied were not pigmented, there is no reason to feel that there was, at some time before their eruption, prolonged elevation of serum levels of bile pigments or porphyrins. That unerupted teeth of patients with severe neonatal jaundice may be stained green, and that teeth of patients with porphyria erythropoietica are red or brown, is well known.[11, 13] The iron content of teeth of such persons is not known. These phenomena demonstrate that chemical entities other than normal components of teeth can be deposited in teeth during their period of formation, and lend support to the possibility that the excess iron in the teeth of the patient under discussion was deposited before their eruption.

SUMMARY

Analyses for iron were performed on 2 carious teeth from a girl who received many transfusions, totalling approximately 21,600 c.c. of blood (11 Gm. of iron) over a 7-year period, and who developed transfusional hemosiderosis. The teeth studied contained an average of 50.8 μg of iron per gram of tooth, whereas the average value of similarly treated carious teeth from control patients was 16.1 μg per gram. Whether the iron was deposited in the teeth before or after eruption is not known. Since salivary glands commonly show hemosiderosis in iron-storage disease, the iron content of saliva in such patients may be elevated, and the teeth may absorb iron from the saliva.

However, it is also possible that the iron was deposited in the teeth before their eruption. Studies on teeth of older persons with acquired hemosiderosis will be necessary to settle this point.

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DISCOLORATION OF TEETH BY METALLIC IONS

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IN RECENT years there has been much evidence showing the presence in enamel of a number of trace elements—copper, iron, fluoride,[1] manganese, silicon, silver, and tin.[2] While their significance has not been established satisfactorily, in vitro studies have shown that exposure of enamel to solutions of some of these elements results in their deposition in the enamel making it more resistant to the action of acids.[3] However, the possible association of some of these elements with tooth discoloration is also of interest because their sulfides are brown or black, and in the oral cavity there exists ample opportunity for the production of these sulfides from food and saliva.

W. D. Miller[4] noted the discoloration of the teeth of men working with copper, iron, lead, and mercury and demonstrated the presence of these elements in the enamel. He also noted the greenish discoloration of teeth which were restored with copper amalgam. Since then, although there has been a great deal of work concerning the distribution and possible significance of naturally occurring green and brown stains,[5, 6] the effect of metallic deposits on enamel has, until very recently, been neglected. A number of elements including copper, iron, lead, silver, and tin have been found in green or brown stains.[7] Unfortunately, in this study no reference was made to the possible presence of these elements in unstained enamel from the same teeth.

The current use of tin in combination with fluoride for "topical" applications and in dentifrices has caused some concern as to the possibility that it may stain enamel. Shapiro and Hood[8] were able to produce marked staining of the enamel, dentin, and cementum in vivo by treating teeth with a 0.4 per cent solution of stannous fluoride and subsequently painting them with an aqueous solution of hydrogen sulfide. Thus, there is some evidence to show that several metallic ions may cause discoloration of teeth. With this in mind, an investigation was undertaken to compare the possible staining properties of lead, tin, silver, mercury, copper, and iron when deposited in enamel and subsequently exposed to a sulfur-containing medium.

MATERIALS AND METHODS

Two solutions containing a cation concentration of 1,000 ppm and 100 ppm were prepared from each of the following salts: cupric sulfate, ferric nitrate, stannous chloride, silver nitrate, mercuric chloride, and lead nitrate. In order to make comparisons at the same pH, similar solutions of each salt were made up at pH 4, using 1 M acetate buffer.

Twelve sound premolars, extracted for orthodontic reasons, were stored in a refrigerator until ready for use. Each tooth was cut longitudinally into

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4 sections with a diamond wheel. The cut surfaces of each section were then covered with wax so that in each case only the external surface of the crown and root was left exposed. The 4 quarters of each tooth were differentiated from each other by using waxes of different colors. The teeth were treated in the following way: One quarter was placed in a test tube with 20 c.c. of the unbuffered solution of the cation and shaken for 15 minutes. The next quarter was exposed in a similar manner to the buffered solution of the cation and the third quarter was etched for 10 minutes in 1 M acetate buffer pH 3 before exposure to the unbuffered solution of the cation. This was done to simulate permeable areas found clinically on teeth, e.g., white spots and eroded, abraded, and partially decalcified areas. The last quarter of each tooth which was used as a control was exposed to distilled water. After exposure to the respective solutions, each quarter was shaken in 20 c.c. of distilled water for 30 minutes and then brushed for half a minute with a rotating bristle brush and distilled water in order to remove any free solution on the tooth surface. The teeth were then placed in a test tube of distilled water through which was bubbled a constant stream of hydrogen sulfide for 15 minutes to favor formation of the sulfide of each cation. After the amount of discoloration caused by the sulfide had been recorded, the teeth were brushed with pumice and distilled water for 1 minute and then re-exposed to hydrogen sulfide. The discoloration produced at this time gave a rough estimation of the degree of penetration of the cations.

Changes in color were gauged visually by comparison with the control as follows: 0 no change, + slight staining, ++ marked staining, and +++ very heavily stained. In addition, a photometer (Spectra Brightness Spot Meter) was used as a check on the visual evaluation.

The distance between the teeth, the photometer and the light source was standardized as follows: The root tips of the 4 quadrants of each tooth were set side-by-side in plaster on an acrylic base which was mounted on a fixed stand. This stand was placed at a fixed distance from the photometer which could be rotated both vertically and horizontally for focusing. To ensure standard lighting the room was completely darkened except for 2 lamps which were placed at a fixed distance from the photometer and connected with a rheostat and voltmeter so that the voltage could be kept constant. With this arrangement the teeth could be removed, treated, and repositioned for color measurements under standard conditions. In order to check the photometer before use and to make sure that conditions remained standard throughout the experiment readings were taken of a white card placed at a fixed distance from the photometer. The card readings remained constant at 150 foot lamberts throughout the experiment.

Three groups of readings of the crowns and roots were recorded. The first group of readings was taken before the teeth were exposed to their respective solutions. In the case of the pre-etched teeth readings were taken after the etching. The second group of readings was taken after the teeth were exposed to the hydrogen sulfide. The final group of readings was taken after the teeth were brushed with pumice and re-exposed to hydrogen sulfide.

RESULTS

The results obtained by visual inspection are tabulated in Table I. Those obtained with the use of the photometer are tabulated in Table II. These two sets of results substantiate each other. Discoloration was apparent on all the crown and root surfaces which were treated with the various solutions while the controls remained unchanged. The root surfaces in most cases stained more heavily than the crowns.

TABLE I

VISUAL ASSESSMENT OF DISCOLORATION PRODUCED BY EXPOSURE TO CATIONS AND HYDROGEN SULFIDE

	$Pb(NO_3)_2$					$SnCl_2$					$CuSO_4$				
		CROWN		ROOT			CROWN		ROOT			CROWN		ROOT	
	pH	A*	B†	A	B	pH	A	B	A	B	pH	A	B	A	B
1,000 ppm of Cation															
Unbuffered	4.9	++	+	+++	+	2.5	+++	+	+++	+	4.4	+	+	++	0
Buffered	4.0	+++	+	+++	0	3.8	++	+	+++	+	4.0	+	+	++	0
Pre-etched	4.9	+++	+	+++	+	2.5	+++	+	+++	+	4.4	+	+	++	0
100 ppm of Cation															
Unbuffered	5.1	+++	+	+++	0	3.1	++	+	++	+	4.9	+	0	++	0
Buffered	4.0	+++	+	+++	0	3.9	+	+	++	+	4.0	+	0	++	0
Pre-etched	5.1	+++	+	+++	0	3.1	++	+	++	+	4.9	+	0	++	0

	$HgCl_2$				$Fe(NO_3)_3$					$AgNO_3$				
		CROWN		ROOT			CROWN		ROOT			CROWN		ROOT	
	pH	A	B	A	B	pH	A	B	A	B	pH	A	B	A	B
1,000 ppm of Cation															
Unbuffered	4.5	++	+	+++	+	2.5	+++	+	+++	0	5.0	+++	+	+++	+
Buffered	4.0	++	+	+++	+	4.0	+++	+	+++	0	4.0	+++	+	+++	+
Pre-etched	4.5	++	+	+++	+	2.5	+++	+	++	0	5.0	+++	+	+++	+
100 ppm of Cation															
Unbuffered	4.8	++	+	+++	+	3.0	+++	+	+++	+	5.2	+++	+	+++	+
Buffered	4.0	++	+	+++	+	4.0	+++	+	+++	+	4.0	+++	+	+++	+
Pre-etched	4.8	++	+	+++	+	3.0	+++	+	+++	+	5.2	+++	+	+++	+

0 = no change; + = slight staining; ++ = marked staining; +++ = very heavily stained.
*Columns A = discoloration of intact surfaces.
†Columns B = pumiced surfaces re-exposed to hydrogen sulfide.

The greatest discoloration was observed in the sections treated with the unbuffered solutions of stannous chloride (pH 2.5) at a concentration of 1,000 ppm and with the buffered solutions of ferric nitrate (1,000 ppm and 100 ppm). When the pH of the solutions of stannous chloride at concentrations of 1,000 ppm (pH 2.5) and 100 ppm (pH 3.1) was raised to about pH 4.0, there was a considerable decrease in the intensity of the staining produced. This may be a reflection of a decrease in the amount of available tin due to the precipitation of insoluble tin compounds such as stannous hydroxy chloride, when the pH is raised to 4.0. It has been shown that less tin is taken up at high as compared to low pH.[9] At pH 4.0, the solutions of ferric nitrate, lead nitrate, and silver nitrate produced a greater discoloration than stannous chloride, mercuric chloride, and cupric sulfate.

An increase in the degree of discoloration was frequently evident when the sections were pre-etched. Apparently the etched areas were capable of taking up greater amounts of the metallic cations. In additional tests which

TABLE II

DISCOLORATION PRODUCED BY EXPOSURE TO METALLIC CATIONS MEASURED BY PHOTOMETER; FIGURES REPRESENT NUMBER OF FOOT LAMBERTS OF REFLECTED LIGHT

	$Pb(NO_3)_2$							$SnCl_2$							$CuSO_4$						
		CROWN			ROOT				CROWN			ROOT				CROWN			ROOT		
	pH	A*	B†	C‡	A	B	C	pH	A	B	C	A	B	C	pH	A	B	C	A	B	C
1,000 ppm of Cation																					
Control		85	85	90	80	80	80		130	130	135	125	120	125		90	90	90	90	90	95
Unbuffered	4.9	110	85	90	90	35	70	2.5	120	40	90	150	50	130	4.4	130	90	120	100	40	100
Buffered	4.0	100	55	90	100	30	90	3.8	110	75	100	120	20	90	4.0	110	70	90	100	40	90
Pre-etched	4.9	120	40	70	110	15	100	2.5	125	40	100	125	30	110	4.4	100	60	85	100	20	100
100 ppm of Cation																					
Control		110	105	110	95	95	100		120	120	125	100	100	100		100	100	100	70	70	80
Unbuffered	5.1	150	90	140	100	30	100	3.1	100	70	85	100	60	90	4.9	130	120	130	100	70	90
Buffered	4.0	130	70	110	95	30	90	3.9	95	80	80	80	50	60	4.0	125	100	105	100	50	90
Pre-etched	5.1	140	50	100	110	30	110	3.1	120	90	100	130	90	110	4.9	100	70	80	90	30	80

	$HgCl_2$							$Fe(NO_3)_3$							$AgNO_3$						
		CROWN			ROOT				CROWN			ROOT				CROWN			ROOT		
	pH	A	B	C	A	B	C	pH	A	B	C	A	B	C	pH	A	B	C	A	B	C
1,000 ppm of Cation																					
Control		120	120	125	105	105	110		120	120	125	125	125	130		120	120	130	130	130	130
Unbuffered	4.5	125	75	90	90	40	50	2.5	130	60	80	125	70	100	5.0	140	90	125	130	30	110
Buffered	4.0	120	90	95	100	55	80	4.0	125	50	60	125	70	100	4.0	125	70	100	100	30	100
Pre-etched	4.5	140	80	100	110	40	75	2.5	140	45	100	125	110	120	5.0	110	60	100	100	50	90
100 ppm of Cation																					
Control		100	95	100	80	75	80		135	135	145	135	130	140		130	125	130	120	115	125
Unbuffered	4.8	130	95	110	110	50	70	3.0	140	70	110	135	50	80	5.2	130	95	110	130	50	100
Buffered	4.0	100	85	95	100	50	70	4.0	135	50	100	120	40	90	4.0	140	80	100	130	70	90
Pre-etched	4.8	125	75	90	110	50	85	3.0	140	70	100	125	40	85	5.2	145	90	100	125	60	95

*Columns A = original reading.
†Columns B = reading of intact surface after exposure to solution and hydrogen sulfide.
‡Columns C = reading after surfaces pumiced and re-exposed to hydrogen sulfide.

included teeth with carious lesions, it was found that the staining of white spots and defective areas was more pronounced than that of sound enamel.

When the crown and root surfaces were brushed with pumice, most of the stain was removed. When they were re-exposed to hydrogen sulfide, slight staining reappeared. It appears, therefore, that although the uptake of the cations was mainly confined to the surface there was some penetration inward.

DISCUSSION

Under the conditions of this experiment, all the metallic cations tested were taken up by the crown and root surfaces of the teeth and produced discoloration when the teeth were subsequently exposed to a sulfur-containing medium. This was apparent when the cation concentration was as low as 100 ppm in the solutions used. Our method therefore appears to be suitable for the detection, in vitro, of the uptake of many metals by tooth structures.

Since metallic sulfides formed readily in the test tube, it is likely that they will also form in the mouth if sulfide is available. This, indeed, appears to be true. Intra-oral sulfide may be released from the thiocyanate present in saliva. Sulfides may also be formed from the available sulfur in foods and from the sulfurous acid which is used as a preservative in meats and dried fruit.[10] Moreover, particularly in cases of poor oral hygiene, retained food debris could provide a suitable substrate for the production of hydrogen sulfide by certain strains of oral microorganisms.

In view of the fact that the daily diet contains approximately 0.29 mg. lead, 17.14 mg. tin, 2.32 mg. copper,[11] and 12.0 mg. iron,[12] one would expect that these elements are incorporated in the enamel during its formation and that they accumulate in increasing amounts with age. Variations in the natural color of teeth of different individuals and the darkening of teeth associated with aging may well be a reflection of the concentration of the metallic elements in enamel. It has been shown, for example, that there is a wide variation in the copper content of enamel from different teeth.[13] It has also been shown that the concentration of lead increases with age.[14] However, this is not true of most of the other trace elements. They are all present in enamel but with the exception of lead and iron their concentrations in teeth of young as well as old persons is very low. It does not necessarily mean that these elements will not react readily with tooth structures; they may rather be present in the oral environment in un-ionized unreactive forms. This is true, for example, in the case of tin which forms insoluble complexes at the pH normally occurring in the mouth. Obviously, factors such as a suitable pH and the availability of the particular element in food as well as in blood and saliva must influence its deposition in enamel.

The incorporation in the enamel of several of the metallic elements increases its resistance to the dissolving action of acids.[3] The sulfides of these elements are very inert and may therefore provide the enamel with a protective coating. The observation[15] that the use of stannous fluoride topically is more effective than sodium fluoride in reducing the incidence of dental

caries in children is supported by this evidence. Solutions of stannous fluoride, with a tin concentration of approximately 15,000 ppm and a pH of 2.9, have been used for topical applications while the stannous fluoride dentifrice in current use contains approximately 3,000 ppm tin and has a pH of about 5.3. Deposition of tin in enamel has been shown after exposure both to the stannous fluoride solutions and the stannous fluoride dentifrice.[19] It is therefore not surprising that staining of the enamel has been associated with the use of stannous fluoride solutions for topical applications. According to our findings this staining would be accentuated in demineralized areas.

SUMMARY

1. The crown and root surfaces of intact teeth were treated with solutions of cupric sulfate, ferric nitrate, stannous chloride, silver nitrate, mercuric chloride, and lead nitrate and subsequently exposed to a sulfur-containing medium.

2. The formation of a dark metallic sulfide in each case indicated that all the metallic cations were taken up by the tooth structures.

3. The degree of discoloration gauged by inspection was substantiated by quantitative readings taken with a photometer which measured directly in foot lamberts the amount of light reflected from a given surface.

4. The degree of discoloration was affected by the pH of the solutions used and was generally more marked on the root surfaces than on the crowns. Areas which had been pre-etched showed a greater tendency toward marked discoloration.

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EFFECT OF NITRIC ACID AND CHELATION DEMINERALIZATION ON VARIOUS STAINS OF HISTOCHEMICAL NATURE

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THIS study was undertaken to compare the effect of nitric acid demineralization and chelating with ethylenediamine tetraacetic acid (hereafter referred to as EDTA) on the staining reactions of hematoxylin and eosin, Feulgen Schiff, thionin, and azure "B."

Histologic studies of mature hard tissues, bone and teeth, have been conducted for the most part with formalin fixation and acid demineralization. Histochemical studies on demineralized material have been limited because acid demineralization interfered with the staining.[1-4] Recently the sodium salts of the organic chelating agent EDTA, which removes minerals in an alkaline medium, have been found suitable for the demineralization of hard tissues.[5-12] With the possibility of demineralization in an alkaline medium, it seemed advisable to investigate what effect demineralization with EDTA would have on the histochemical stains often used in histochemical analysis, namely, Feulgen Schiff, thionin, and azure "B."

MATERIALS AND METHODS

To be sure that any conclusions regarding the effect of chelation were not due to the formalin fixation, the frozen dried method which is readily accepted for processing tissues for histochemical studies was used as a control. This necessitated using a soft tissue which could be frozen and dried, or formalin fixed. Rat cerebellum, in spite of its difficult fixation under any conditions, was selected because nerve tissue has been the subject of many histochemical studies,[13-15] and the Purkinje cell, being a large cell, could be readily identified.

Cerebellums of young rats were subjected to one of the following treatments:

1. Freezing in isopentane which had been chilled with liquid nitrogen to –130° C., dehydration in vacuuo at –30° to –40° C., embedding in paraffin and cutting and dry mounting according to the standard method of Gersh.[16] Sections were postfixed overnight in absolute alcohol to avoid the dissolution of some protein containing compounds that might be lost during the staining procedure.

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TABLE I
STAINING OF PURKINJE CELLS OF RAT CEREBELLUM FOLLOWING VARIOUS METHODS OF PREPARATION

METHODS OF PREPARATION	STAINS AND STAINING REACTION				CELLULAR DETAIL
	HEMATOXYLIN-EOSIN	FEULGEN SCHIFF	THIONIN	AZURE "B"	
	Nucleus—Blue Nucleolus—Purple Cytoplasm—Pink	Nucleus—Reddish Purple Nucleolus—Colorless Cytoplasm—Green*	Nucleus—Blue Nucleolus—Purple Metachromasia Cytoplasm—Purple Metachromasia	Nucleus—Bluish Green Nucleolus—Purple Metachromasia Cytoplasm—Purple Metachromasia	
Frozen and dried and formalin fixed; embedded in paraffin	No difference in reaction or intensity	No difference in reaction; intensity greater with formalin fixation	No difference in reaction; intensity greater with formalin fixation	No difference in reaction; intensity greater with formalin fixation	More morphologic detail with formalin fixation
Formalin fixed untreated; treated with buffered saturated solution of disodium salt of EDTA pH 7.4 for 10, 30, and 56 days; embedded in paraffin	Reaction and intensity same in untreated and treated with EDTA; time not a factor	Reaction and intensity same in untreated and treated with EDTA; time not a factor	Reaction same in untreated and treated; intensity of color decreased with increase in time of treatment —30 and 56 days difficult to interpret	Reaction same in untreated and treated; intensity of color less in EDTA treated material but interpretation still possible after 30-56 days	Good morphologic detail in untreated and EDTA treated material
Formalin fixed; treated with 5% nitric acid 3 days; embedded in paraffin	Over-all eosinophilia	Negative	Negative	Negative	Less morphologic detail than in untreated and EDTA treated sections
Embedded in paraffin; embedded in celloidin	Reaction and intensity the same in both embedding media	Satisfactory in paraffin; reaction and intensity variable with celloidin embedding	Reaction same but intensity greater in celloidin embedded untreated sections; satisfactory in paraffin; unreliable in EDTA treated material embedded in celloidin	Satisfactory in paraffin; reaction not consistent in celloidin embedded material	Same morphologic detail in both embedding materials when stained with H&E; other stains did not permit comparisons

*Counterstain—Fast Green (without counterstain cytoplasm is colorless).

2. Fixation in 10 per cent neutral formalin for at least 2 days, dehydration in the alcohols, embedding in paraffin or in celloidin.

3. Fixation in 10 per cent neutral formalin for at least 2 days, suspension at room temperature in a saturated solution of the disodium salt of EDTA made up in phosphate buffer to pH 7.4 for periods of 10, 30, and 56 days, dehydration in the alcohols, embedding in paraffin and/or in celloidin.

4. Fixation in 10 per cent neutral formalin for at least 2 days, suspension at room temperature in 5 per cent nitric acid for 3 days, dehydration in the alcohols, embedding in paraffin and/or in celloidin.

All sections were cut at 10 microns and stained with the same dye solution, dehydrated, and mounted in terpene polymer resin, Hartman Leddon, "H.S.R." The stains used were alum hematoxylin and alcoholic eosin, Feulgen Schiff[17] counterstained with fast green,[18] thionin[19] modified by LaVelle,[14] and azure "B."[20]

Koehler illumination was used in the microscopic analysis and, for metachromasia, the slides were examined with yellow light. The sections were examined for staining reaction and intensity as well as the cellular detail of the Purkinje cells.

RESULTS

See Table I.

DISCUSSION

I. *Comparison of Frozen and Dried and Formalin-Fixed Material Embedded in Paraffin.*—

According to Gersh,[16] the frozen dried method of preparing tissue for histologic studies preserves both the morphology and chemical component of the cell more truly than chemical fixation. All known methods of demineralization use a chemical fixative, the most common being formalin. Crawford and Barer[21] compared formalin-treated cells with living cells using phase contrast microscopy, and found that neutral formalin produced far less visible alteration of cytoplasm and nuclear texture than the majority of other fixatives with the exception of osmium tetroxide. In this study, the frozen and dried material was used as a standard for comparing the formalin-fixed tissue before evaluating the staining results following demineralization.

The staining reaction in both methods of tissue preparation was the same, but the intensity of the stain with Feulgen Schiff, thionin, and azure "B" was greater in the formalin-fixed specimens than in those specimens frozen and dried. Brachet[22] stated that it had not been possible to obtain really brilliant basophilia using the frozen and dried method, and suggested that the presence of lipids which are not necessarily eliminated by the frozen and dried technic might explain the difference in staining results. It is also possible that the formalin changes the chemical composition of the cell in such a way that there were more groups available to react with the stain, which might increase the intensity.

Nevertheless, the results in this study indicate that neutral formalin as a fixative does not interfere with Feulgen Schiff, thionin, or azure "B" staining. The reason for the increase in staining intensity with formalin fixation merits further study.

Cellular detail: The more definite detail in cellular morphology observed in the formalin-fixed material may not be considered desirable by all investigators. Many histologists are convinced that the cell processed by the frozen dried method gives a truer picture than one chemically fixed, and that the more clearly defined structures are artifacts introduced by the chemical fixative. Regardless of whether or not this detail is an artifact, studies of hard tissue which must be demineralized before sectioning require chemical fixatives. Results of formalin-fixed material may be interpreted with this in mind.

II. *Comparison of the Formalin-Fixed Paraffin Embedded Material Which Was Either Untreated, Treated With EDTA, or With Nitric Acid.*—

The difference in the staining results of Feulgen Schiff, thionin, and azure "B" following the 2 methods studied may be explained by the difference of the pH of the solutions and the more drastic chemical reaction of the acid on the nucleic acids. Lillie,[23] in studying the effect of pH on staining of bone marrow, noted that soaking bone marrow in buffered mixtures of sulfurous acid and formic acid of pH 1.15 to 1.30 for only 1 day destroyed the basophilia of nuclear chromatin. Later[1] he suggested that ". . . it appeared that strong acids must dissolve, hydrolyze or denature the nucleotid phosphoric acid ribose and desoxyribose complexes." Nikiforuk and Sreebny[7] observed that EDTA removed calcium from bone most efficiently near a neutral pH. They also noted a drop in pH as the demineralization proceeded. Potassium phosphate sodium hydroxide buffer was used in this study to avoid the change in pH, but it was found to be unsatisfactory. Hahn and Reygadas[5] neutralized their chelating solution with sodium pyrophosphate to maintain the pH level, and observed that the rate of the reaction was increased; but they did not check staining results.

Until further study can be made on a more efficient buffer for the chelating agent EDTA and the effect on staining reactions, it seems advisable that the EDTA be made in an aqueous solution and the pH adjusted to the desired level and checked daily. When a pH drop occurs the specimens should be placed in a fresh solution.

Stains: Feulgen Schiff: The Feulgen Schiff reaction is generally accepted as a histochemical stain specific for desoxyribonucleic acid. The positive result obtained with this reaction in the formalin-fixed material both untreated and treated with EDTA for 10, 30, and 56 days indicates that the desoxyribonucleic acid is not visibly altered by the chelating solution beyond any change which may have occurred during fixation.

Thionin and Azure "B": Some of the ribonucleic acid is undoubtedly extracted from the tissue by the chelating solution, since there is less intense metachromatic staining of the chelated material. However, the results indicate that demineralizing with EDTA does not destroy the ribonucleic acid, as that

which remains following demineralization shows the same, though less strong, staining reaction as the undemineralized material. This is an advantage over nitric acid demineralization, and the results may be interpreted with this in mind. The loss of ribonucleic acid may be partially overcome by reducing the period of demineralization to a minimum.

III. *Comparison of the Paraffin and Celloidin Embedded Material.*—

Many investigators in studying demineralized hard tissues prefer celloidin embedding to paraffin because they feel that it maintains more adequately the relationship of the hard and soft tissues.

Since both methods of embedding give comparable results with hematoxylin and eosin staining, dependent upon the method of demineralization, either embedding method may be used. When using Feulgen Schiff, thionin, or azure "B" on EDTA demineralized tissue, alcohol storage of celloidin sections tends to bring about a loss of color in the staining, especially of thionin. When chelated celloidin sections are stained with thionin immediately after cutting, the results are satisfactory. However, after 2 days' storage in 75 per cent alcohol, the stain is so faint that it is difficult to evaluate the results. Brachet[22] stated that for some unknown reason prolonged contact with alcohol reduces the affinity of nucleic acids for basic dyes. Paraffin sections, however, may be stored dry. For studies using Feulgen Schiff, thionin, or azure "B" on EDTA demineralized material, paraffiin embedding is recommended.

SUMMARY

Comparisons were made of the effect of fixation (frozen and dried and formalin), the effect of demineralizing treatment (ethylenediamine tetraacetic acid and nitric acid) and the effect of embedding (paraffin and celloidin) on rat cerebellum stained with hematoxylin and eosin, Feulgen, Schiff, thionin, and azure "B."

The staining reaction was similar in both methods of fixation. The staining intensity was greater with Feulgen Schiff, thionin, and azure "B" in formalin fixation and there was more definite detail of cellular morphology with formalin fixation.

Specimens treated with EDTA gave characteristic staining reactions to hematoxylin and eosin, Feulgen Schiff, thionin, and azure "B," although the staining was less intense with thionin and azure "B" than in the untreated material.

Sections stained with hematoxylin and eosin, following treatment with nitric acid, showed an over-all eosinophilia, and those stained with Feulgen Schiff, thionin, and azure "B" were colorless.

Both paraffin and celloidin gave comparable results when the chelated material was stained with hematoxylin and eosin, but paraffin was more satisfactory than celloidin when material which had been treated with EDTA was stained with Feulgen Schiff, thionin, and azure "B."

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ENAMEL CARBONATE IN CARIES

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THAT, at an early stage in caries, the enamel carbonate is preferentially lost has been inferred by Hardwick[1] from analyses of acid solutions which have acted on teeth and by Gore[2] from analyses of powdered enamel treated with acid solutions containing calcium and phosphate.

The following analyses of early carious enamel demonstrate that CO_2 is preferentially lost in early clinical caries.

EXPERIMENTAL

The teeth were all from adults being treated for a variety of conditions. Normal enamel was ground with a diamond wheel from the surface of teeth previously cleaned with a rubber buffing wheel.* Enamel was removed by an explorer from fresh, smooth surface lesions of the "white spot" type showing unchanged surface contour and not affecting the dentin, and from artificially altered enamel produced in experiments previously described.[4]

All the clinically carious and artificially altered enamel (between 0.5 and 2.0 mg.) removed from each tooth was transferred quantitatively to a glass vessel of 10 ml. capacity and allowed to remain in the microbalance case until it had attained a constant weight. (The weight of normal enamel did not change.) Carious enamel lost about 10 per cent of its initial weight in about 12 minutes. (One sample of carious enamel which had thus lost 10 per cent of its initial weight was heated 18 hours at 110° C. and lost no additional weight.) Into the 10 ml. vessel containing the sample was then placed a small glass dish of 1.0 ml. capacity, coated with paraffin. The 2 vessels were placed overnight inside a third vessel containing NaOH pellets and closed with a paraffin lid. In the morning the paraffin lid was pushed down inside the outer vessel and sealed to the rim of the 10 ml. vessel containing the 1 ml. dish and the sample, which were thus sealed in a CO_2-free atmosphere. Into the dish was then pipetted, through the paraffin lid by means of a Krogh-Keyes syringe pipette with a No. 22 stainless steel needle, a precisely determined amount (approximately 0.2 ml.) of standard, approximately 0.02 M, $Ba(OH)_2$ solution containing thymol blue. Two-tenths milliliter of 1 N HCl was similarly introduced around the powdered enamel. The holes in the paraffin lid were sealed with melted paraffin after each addition. The vessels were rocked for 2 hours to

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*Weighings of the diamond wheel between grindings showed that it lost only a negligible amount of weight when grinding enamel. Previous analyses by one of us (T.B.C.) of normal enamel ground from teeth with a carborundum wheel[3] are probably in error because of contamination of many of the samples with carborundum.

allow passage of CO_2 into the $Ba(OH)_2$ solution, which was then titrated together with a control, treated as above but without enamel, with 0.1 N HCl by means of a Gilmont ultramicro buret of 0.1 ml. capacity. Following the titration of the CO_2, the CO_2-free enamel powder solution was made up to 10 ml. From this solution were taken duplicate 4.0 ml. aliquots which, after passage through 1 cm. by 7 cm. columns of Amberlite IR4B-OH* followed by 16 ml. of distilled water were titrated with versene after the addition of 1 ml. of 1 N NaOH and 1 ml. of calcium indicator solution.† Five-tenths milliliter aliquots of the enamel solution were analyzed for phosphorus by the method of Gomori.[6]

RESULTS

The results of all the analyses of normal and of clinically carious enamel are given in Table I, together with the standard error. For comparison, the macro-analyses of Armstrong and Brekhus[7] for whole enamel separated by flotation and the macro-analyses of Bowes and Murray[8] for enamel removed by grinding from the tooth surface with a steel wheel are also given. The results indicate that the composition of enamel obtained by grinding from the surface is different from that of whole enamel separated by flotation.

TABLE I

	CALCIUM (%)	PHOSPHORUS (%)	CO_2 (%)	CALCIUM : PHOSPHORUS	CALCIUM CO_2
Normal enamel removed from tooth surface with diamond wheel; 12 micro analyses	37.9 ±2.8	17.3 ±0.8	1.92 ±0.2	2.23	21.2
Carious enamel "white spot" without loss of surface contour, removed with explorer; 8 micro analyses	30.9 ±4.4	13.4 ±1.5	1.04 ±0.2	2.34	33.2
Artificially altered enamel, same hardness as clinical caries, Expt. F3A	24.7	12.6	1.06	1.95	23.3
Artificially altered enamel, soft, Expt. F3AB	27.1	13.3	1.53	2.04	17.7
Normal enamel, whole enamel separated from dentin by flotation; 40 macro analyses; Armstrong and Brekhus[7]	35.4	17.5	3.0	2.03	11.8
Normal enamel, removed from tooth surface with steel wheel, several macro analyses; Bowes and Murray[8]	37.3	17.2	1.95	2.15	19.1

The hole left by the removal of enamel from one of the clinical lesions appeared to be of suitable shape for reproducible filling and was carefully filled 5 times with inlay wax. The first 4 fillings were weighed. All weights were within 1 per cent of the mean. The fifth filling was left in place while the tooth was sectioned under cold water and was seen to fill the hole accurately. From the density of the wax and the weight of the filling, the volume occupied by the carious enamel in situ was calculated. From this volume, from the

*Analytical grade. Fisher Scientific Company. Washed with 4 per cent sodium acetate solution followed by water.

†Code No. 293—W. H. and L. D. Betz Company, Philadelphia, Pa.[5]

density of normal enamel,[9] and from the composition of the normal enamel by analysis, the amounts of calcium, phosphorus, and CO_2 originally present in the volume were calculated. From these values and from the analysis of the carious material, a calculation was made of the calcium, phosphate, and CO_2 lost in the carious process, estimated as percentages of the amounts originally present. The calculation indicates that 40 per cent of the calcium, 41 per cent of the phosphorus, and 66 per cent of the CO_2, originally present in the normal enamel, had been lost when the carious process had reached the "white spot" stage. While only one calculation of this kind was made, the uniformity of composition and of hardness of carious enamel at this stage lead us to believe that our calculation of the fraction of each constituent lost probably lies fairly close to the general value for this type of lesion.

Several samples of enamel altered by treatment with chemical solutions were analyzed. Only one of these samples (from Experiment F3A) resembled the clinically carious material in hardness. The analysis of this sample is given in Table I. The other samples of artificially altered enamel varied in composition but in general they did not show a greater loss of CO_2 than of calcium and phosphate. The analysis of the enamel from Experiment F8AB is typical of the softer, artificially altered enamel. It can be concluded from the analysis of the clinical lesions and of the artificially altered enamel that the conditions under which the CO_2 loss demonstrably exceeds the calcium and phosphate loss are narrowly limited.

The clinically carious and artificially altered enamel removed by the explorer was shown by examination of ground sections to have come from positions showing zones E and F of Nishimura.[10]

DISCUSSION

The inorganic material of enamel is chiefly crystalline hydroxylapatite with isotropic inorganic material between the crystals. The carbonate in enamel has not been shown to be present as calcium carbonate and it is not certain that any of the carbonate is present in the crystal lattices of hydroxylapatite. Chase[11] observed, under the microscope, that the carbon dioxide escaping from ground sections during acid decalcification appeared to come from the inter-rod spaces and from the rods themselves.

The preferential removal of CO_2 shown by analysis above is probably not maximal in zones E and F, where considerable solution of the apatite crystals themselves has occurred. It seems reasonably certain that, in the deeper zones (A, B, C, and D), the ratio of carbonate loss to calcium and phosphate loss is higher than has been demonstrated for zones E and F. The demonstration of such a loss of carbonate in the deeper zones by the analytical methods described here would be difficult.

SUMMARY

Analyses show that carbonate is lost faster than calcium and phosphate in early "white spot" clinical caries.

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THE PRODUCTION OF ACIDS FROM GLUCOSE BY ORAL MICROORGANISMS: ACIDS OF THE KREBS CYCLE

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THE question of acids produced in the oral cavity from the action of oral microorganisms on carbohydrates has been briefly reviewed in previous papers from our laboratory.[1, 2]

Burrows[3] lists several microorganisms present in the oral cavity which utilize the Krebs cycle as a main oxidative pathway. With this in mind, the question is raised as to whether all of the organic acids present in the tricarboxylic acid cycle appear when unfiltered saliva is incubated with glucose.

MATERIALS AND METHODS

Saliva was obtained by paraffin stimulation and incubated with glucose at 37° C.[1] Samples of saliva were obtained from 42 individuals, mostly adults who clinically were either caries active or caries inactive. They were about equally divided in these 2 groups. (One subject was caries immune.)

The acids of the tricarboxylic acid cycle, produced as the result of incubation of unfiltered saliva with glucose, were identified by means of the paper chromatographic procedure of Lugg and Overell,[4] modified by carrying out the determination in a sealed metal chamber, rather than in one of wood. Viewing ports were provided at the side of the chamber to enable the experimenter to determine the extent of advance of the solvent front. As a solvent, N-butanol-5M aqueous formic acid was used.[4] Whatman No. 1 paper strips 1½ inches wide were utilized in the tank. Samples of the solutions under examination were applied at one end of the paper until a circle with a diameter of about ¾ inch was moistened. The paper was then dried and the procedure repeated until a total of 0.25 ml. had been deposited. The strips were air-dried for several hours and subsequently placed in the developing chamber. When the mobile phase had progressed a sufficient distance, the strips were taken from the tank and again air-dried. They were then developed by spraying with a 0.04 per cent solution of bromphenol blue, prepared as suggested by Lugg and Overell.[4] The acids in question were resolved as yellow spots on a pale blue background.

Acid production was noted by comparing the chromatograms of the incubated control solutions with those of the incubated experimental solutions. Approximations of the differences in the quantities of acids produced were made by comparison of the size and color intensity of the spots.*

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*All salivary samples were tested for the presence of coenzyme A by the method employed by Stadtman.[5] All were found to contain detectable amounts of this enzyme.

RESULTS

Comparison of the chromatograms of the control with those of the experimental solutions showed that within 30 minutes after the addition of glucose to unfiltered saliva, detectable quantities of organic acids of the Krebs cycle were found.

Following such incubation with glucose, all salivary samples were shown to produce succinic acid, as well as malic and fumaric acids. Lactic and pyruvic acids were produced in larger quantities than any other acid during the first 30 minutes. Alpha-ketoglutaric acid was produced in all but 2 of the samples, while 39 samples produced oxalacetic acid.*

Confirming previous work,[1] there was no production of citric acid from the action of oral microorganisms on glucose.

Examination of 6 samples which were incubated an additional 30 minutes (making 60 minutes in all) indicated that the concentration of lactic acid reaches a peak during the first 30 minutes of incubation and then decreases sharply, while pyruvic acid concentration increases steadily. This confirms earlier findings.[2] The amount of oxalacetic acid appeared to remain constant, but this may have been due to the acid's tendency toward enolization. All other acids, with the exception of citric acid, increased in concentration as the incubation time increased.

The production of the acids under study after the incubation of unfiltered saliva with gulcose showed no relation to the clinical caries condition of the subjects from whom the saliva samples were obtained.

SUMMARY

1. By the use of paper chromatography, it has been demonstrated that the following acids of the Krebs cycle are produced by the action of oral microorganisms on glucose: malic, oxalacetic, alpha-ketoglutaric, succinic, fumaric, pyruvic, and lactic.

2. The concentration of these acids increases with time, save for lactic and citric acids.

3. The amounts of these acids produced under our experimental conditions bear no relation to the clinical caries picture.

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*Several samples of saliva incubated without glucose showed no such increases in acid production as is herein reported.

REACTION OF TOOTH SURFACES WITH ONE PPM OF FLUORIDE AS SODIUM FLUORIDE

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FLUORIDE is taken up by enamel from the relatively high concentrations of fluoride used in topical applications or in fluoride dentifrices.[1] That fluoride in concentrations of 1 ppm or less also reacts with tooth structure is suggested by the fact that fluoride will accumulate in the outermost layer of the enamel with age when only traces of fluoride are present in the environment.[2] With 1 ppm of fluoride in the drinking water, as much as 1,550 ppm of fluoride may be present in the surface enamel of a person over 50 years of age as compared to only 890 in the surface enamel of a person under 20 years of age.[3] In view of this external fluoride acquisition it is not surprising that posteruptive exposure to 1 ppm of fluoride will reduce dental caries.[1]

The present study was undertaken to provide more information about the reaction between intact enamel and solutions containing 1 ppm of fluoride. Radioisotope technics were adopted which permitted the repeated study of the same area of enamel surface and which allowed measurements of amounts too small for currently available analytical procedures. Our aim was to study the rate of uptake and the extent to which fluoride uptake and removal can be modified by treating the enamel surface.

METHOD

Radioactive fluoride was obtained from the 60-inch cyclotron of the University of Rochester by proton bombardment of water enriched with O^{18}.[4] Decay curves on each sample gave a mean half life of 115 minutes. In no case was extraneous radioactive material detected. The radioactive sample was transferred to a 5 ml. volumetric flask containing 2.5 ml. of 2 ppm fluoride as sodium fluoride. The solution was brought to volume with distilled water and then made up to 25 ml. with 1 ppm of fluoride as sodium fluoride. The teeth were fluoride exposed in Pyrex test tubes containing 5 ml. aliquots of this solution. The test tubes were placed in a rack and fastened to the top of a vibrator.

The teeth to be studied were extracted 1 or 2 days prior to the experiment, from patients of known age and sex. After cleaning, they were coated with wax except for the area to be exposed, and stored on moist cotton in a refrigerator. When 2 or more areas on the same tooth were studied, sections of

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individual areas were prepared. Following the fluoride exposure, the wax was removed and the exposed surface area was washed with a rotating brush in a stream of distilled water to remove the fluoride which had not reacted with the enamel.

Fig. 1 illustrates the counting technic employed. A $\frac{3}{32}$ inch hole was drilled in the center of $\frac{1}{16}$ inch aluminum plates cut to fit the holder beneath the Geiger tube. The surface of each tooth to be studied was adapted to the plate over the hole as follows: Impression compound was heated and dripped around the hole in the plate. A wet piece of Cellophane was placed over the compound and the tooth surface was impressed into the Cellophane so that the desired area was located over the hole in the plate. After cooling,

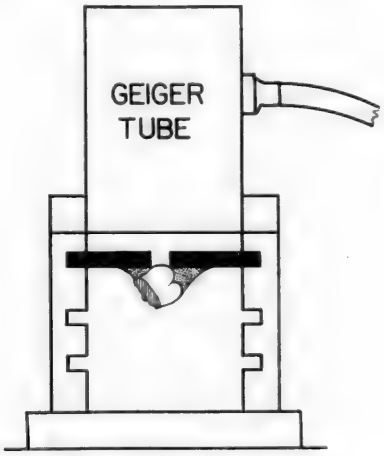

Fig. 1.—Method of counting.

any compound extruding into the hole was removed and the tooth was checked for its fit in the impression. The tooth could be accurately reseated into the impression, permitting repeated counts of the same surface area. A series of tests on 8 teeth showed that counts could be reproduced within 10 per cent accuracy. All counts were calculated back to zero time and, in comparing counts obtained on different radioactive samples, the specific activity was taken into account.

RESULTS

Loss of Fluoride From Enamel Surfaces.—

a. *Effect of brushing with water:* Various washing procedures gave variable results. Brushing by toothbrush in a stream of distilled water for 3 minutes removed from 10 to 50 per cent of the fluoride. Fig. 2 shows the results of experiments in which teeth were fluoride exposed for 5 minutes and counts were taken after successive periods of brushing with a rotating brush in running distilled water. The initial counts, taken after (a) ½ minute, (b) 1 minute, and (c) 2 minutes of washing, are plotted as 100. The curves show that the loss of

fluoride is much more marked in some teeth than in others, demonstrating the different behavior of different teeth. Generally, washing causes a rapid loss of fluoride during the first 3 minutes, and a decreasing loss thereafter. That the residual fluoride may be firmly attached is shown by the fact that even after 8 minutes of brushing, 44 per cent of the fluoride was retained in one case.

b. *Effect of abrasives:* To learn how deeply fluoride penetrated the tooth surface, teeth which had been exposed to fluoride for 5 mintes were pumiced for successive periods, and washed for one-half minute and counted between each pumicing. The pumicing was accomplished by holding the tooth beneath a rubber dental polishing cup attached to an electric drill. A slurry of pumice was kept between the cup and tooth surface during the pumicing.

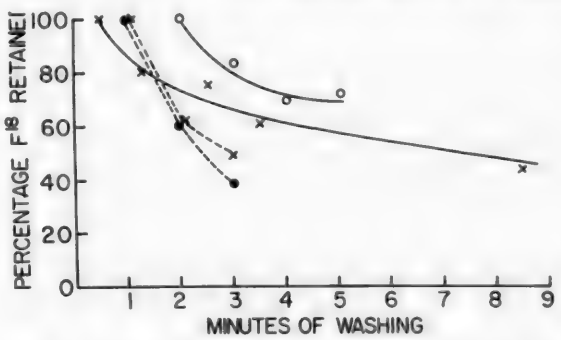

Fig. 2.—Loss of fluoride after scrubbing with water. Initial counts, taken after (a) ½ minute (×——×), (b) 1 minute (●- - -●/×- - -×), and (c) 2 minutes (O——O) of washing, are plotted as 100.

The pressure used during pumicing appeared to be an important factor in removing the flouride. In 8 teeth it was found that 2 seconds of heavy pumicing caused an average of 50 per cent loss (range 35 to 65 per cent), 10 seconds an average of 80 per cent loss (range 65 to 95 per cent), and after 30 seconds only traces of fluoride were left. With light pressure, 4 teeth showed an average of 78 per cent loss (range 63 to 87 per cent) during 1 minute of pumicing.

All abrasives tested removed the greatest amount of the fluoride during the first 30 seconds of brushing. Table I compares brushing for successive periods with slurries of aluminum oxide, perlite, pumice, and calcium carbonate. In these tests the first counts, which were given a value of 100, were taken after

TABLE I
PER CENT FLUORIDE RETAINED AFTER BRUSHING WITH DIFFERENT ABRASIVES

ABRASIVE	PERIOD OF BRUSHING		
	½ MIN.	1 MIN.	1½ MIN.
Aluminum oxide	12	6	2
Perlite	21	10	–
Pumice	32	29	21
Calcium Carbonate	63	55	41

5 minutes of fluoride exposure and a 2-minute washing with brush and distilled water. The marked variations in the effect of the different abrasives may be due to a possible difference in the particle size of the samples tested.

TABLE II
FLUORIDE UPTAKE ON BUCCAL AND LINGUAL SURFACES FROM TEN DIFFERENT TEETH

TOOTH	BUCCAL (COUNTS PER MINUTE)	LINGUAL (COUNTS PER MINUTE)
1	582	330
2	928	706
3	1,637	553
4	595	287
5	264	176
6	282	465
7	229	143
8	160	164
9	185	130
10	718	138
Mean	558	309

Fluoride Uptake by Enamel Surfaces.—

a. *Variations in fluoride uptake:* Counts were obtained on buccal and lingual surfaces of teeth which had been exposed to fluoride for 5 minutes and washed for 2 minutes. Table II shows the wide variation in fluoride uptake between different teeth and between areas on the same tooth. It is of interest to note that in every case except 2, the buccal surface had a greater uptake than the lingual. The mean uptake for the buccal surfaces was 558 counts per minute compared to 309 for the lingual surfaces. In addition, findings in 4 teeth showed from 29 to 52 per cent greater uptake from white or brown spots than from adjacent sound enamel.

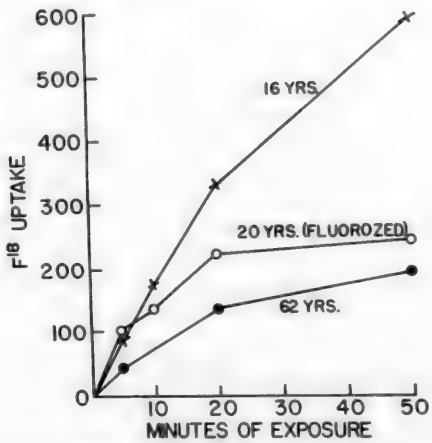

Fig. 3.—Relation of exposure time to fluoride uptake (counts per minute).

b. *Effect of period of exposure:* Teeth were fluoride-exposed for successive periods and after each exposure they were washed for 3 minutes and counted. The results (Fig. 3) show considerable difference in the reactivity of different

teeth. Generally, there was an increase in uptake with time, most marked during the first 10 minutes of exposure. The low pickup on the fluorosed tooth and the oldest tooth examined is of interest in view of the high fluoride content in the surface enamel reported in such teeth.

The reaction of fluoride with teeth appears to take place very rapidly. This was shown in experiments run in duplicate, in which counts were taken after 1, 3, 9, and 20 minutes of exposure, with 2 minutes of washing preceding each count. Twenty-eight per cent of the fluoride acquisition took place during the first minute, 46 per cent during the first 3, and 93 per cent during the first 9 minutes of exposure.

c. *Effect of pumicings:* Because of the common use of abrasives in oral prophylaxis, a detailed study was made of the effect of pumicing on fluoride uptake. Counts were first obtained after 5 minutes of fluoride exposure followed by a 2-minute washing. The teeth were then pumiced for half a minute, washed for half a minute, and again counted. By repeating this procedure of fluoride exposure, washing and counting; pumicing, washing and counting,

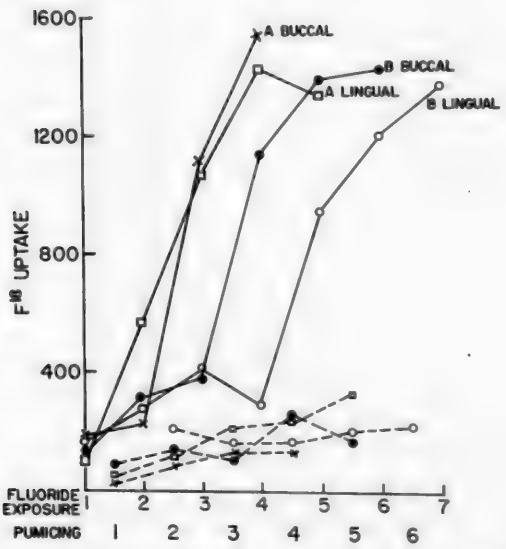

Fig. 4.—Solid curves show fluoride uptake (counts per minute) after repeated pumicings. Broken curves represent radioactivity remaining after each pumicing.

several times on each tooth, it was possible to measure fluoride uptake and loss in enamel at increasing depths from the surface. In Fig. 4, the points on the solid curves represent the count per minute obtained after fluoride exposures. The broken curves represent the radioactivity remaining after each pumicing. There is a markedly greater uptake of fluoride by the pumiced surfaces. An abrupt increase in the rate of uptake occurred in 3of the 4 sections and, in each, a different number of pumicings were required to achieve

the effect. The amount of fluoride remaining after pumicing tended to increase slightly with each fluoride exposure. To what extent this increase represents a greater penetration of fluoride and re-absorption of fluoride from the pumice slurry is not known.

d. *Effect of acid etching:* Counts were obtained of intact surfaces after a 5-minute fluoride exposure followed by a 2-minute washing. The teeth were then etched for 10 minutes in .2 M acetate at pH 4.0 or pH 5.0 and washed, fluoride exposed, washed and counted. The outer enamel was then ground off and, after polishing and washing, the ground surface was fluoride exposed, washed and counted, and etched, fluoride exposed, washed and counted, in the same way as was the intact surface. Etching of the intact surface at pH 4.0 nearly doubled the fluoride uptake while etching at pH 5.0 had no effect (Fig. 5). The ground polished surfaces had more than twice the uptake of the intact surface, again demonstrating the greater acquisition of fluoride by subsurface compared to surface enamel. Etching of the ground surface at both

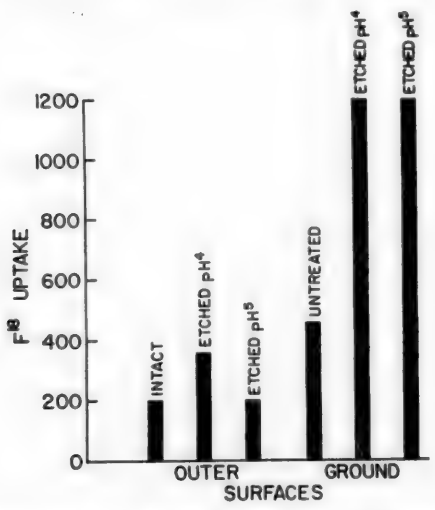

Fig. 5.—Effect of acid etching on fluoride uptake (counts per minute) by intact and ground enamel.

pH 4.0 and pH 5.0 greatly increased the fluoride uptake. Additional tests with acetate and lactate buffers showed that, although there was marked variation in the behavior of different teeth, the uptake by surface and subsurface enamel generally followed the pattern indicated in Fig. 5.

DISCUSSION

It is evident from our findings that fluoride in concentration of 1 ppm will react readily with enamel and that a substantial part of the acquired fluoride is firmly attached to the tooth. This is shown by the fact that several minutes of rigorous brushing with water in most instances removed less than one-half of the fluoride taken up after a 5-minute fluoride exposure. Since

on the other hand most of the fluoride was removed after only a one-half minute brushing with an abrasive, it must be concluded that the fluoride reaction is a surface reaction, with virtually no penetration into the bulk of the enamel. This finding is in agreement with the observation that fluoride normally accumulates in the outer enamel and that there is little increase in the amount of fluoride in subsurface enamel during a lifetime.[2]

Since appreciable amounts of fluoride were taken up after only a 1-minute exposure, one would expect the outer enamel to rapidly assume fluoride saturation in the presence of environmental fluoride. It is interesting therefore that only 1,550 ppm has been found in the outer enamel of teeth from old persons living in areas with 1 ppm of fluoride in the drinking water,[3] compared to the almost 40,000 ppm which theoretically could be present if there were fluoride saturation with complete conversion of the hydroxylapatite to fluorapatite. At least two factors operate to limit the amount of fluoride taken up by the enamel. One is that fluoride and hydroxyl ions compete for a place in the apatite lattice. Therefore fluoride saturation cannot be expected at the nearly neutral pH employed in our study. More important is the fact that while the fluoride ions will react readily with the surface of the apatite crystals in the enamel, penetration into the body of the crystals is either very slow or does not occur. For this reason the amount of fluoride taken up will depend on the extent to which the surfaces of the enamel crystals are accessible to the fluoride ions. Once these surfaces have been saturated, further fluoride increase is likely to result more from slight penetration into the enamel proper than by penetration into the crystals.[2] The fluoride effect may therefore be considered to be a coating effect, which explains how the small amounts of fluoride in the enamel can increase its resistance to dental caries.[5]

It is interesting that fluorosed teeth and teeth from old persons, known to be high in surface fluoride, showed an appreciable uptake in our tests. Furthermore, in experiments not previously mentioned, substantial uptake was obtained with teeth which had been fluoridized by in vitro exposure for 18 or more hours prior to the test. Of 6 tooth halves pre-exposed to nonradioactive fluoride solutions, ranging in concentrations from 1 ppm to 1 per cent, 4 showed higher uptake of radioactive fluoride than the controls, which were exposed to similar solutions of sodium chloride, and the remaining 2 also gave high counts. This suggests that fluoride in the enamel will exchange with fluoride in the solution. In addition to this fluoride with fluoride exchange at least 2 other reactions may take place: One is replacement of hydroxyl groups in the hydroxyl apatite with fluoride ions,[6] the other is adsorption of fluoride to the enamel surface.[4] Fluoride may also enter the enamel by diffusion into cracks and imperfections,[7] a fact which may explain the high counts obtained in such enamel lesions as white and brown spots.

Our finding of increased uptake from pumiced and ground surfaces may be related to several factors. As suggested by Adler,[8] abrasives will remove soft deposits and uncover a more reactive surface. In addition, abrasion and grinding will tend to produce an increase in surface area and thus favor fluoride uptake. Fluoride may also be acquired more readily by subsurface

enamel simply because much less fluoride is present there than in the outermost part of the enamel. Regardless of the cause, it should be noted that the subsurface enamel which is exposed by caries, readily will take up fluoride. Therefore fluoride in the drinking water may slow down the progress of caries by this external fluoride acquisition.

In our tests there was a marked increase in fluoride uptake by both surface and subsurface enamel after etching at pH 4, and only by the subsurface after etching at pH 5. Acid etching, like grinding, may be expected to increase fluoride uptake to the extent it increases the surface area and uncovers subsurface enamel. Since it has previously been shown that surface enamel has a greater resistance to acid than subsurface enamel,[9] the unaltered uptake of the intact surface after etching at pH 5 probably reflects minimal decalcification of the surface at this pH, while the subsurface enamel was decalcified and consequently took up more fluoride.

Considerable variation was obtained in the fluoride uptake by different teeth and different areas on the same tooth. This is not surprising since similar variations have been noted with regard to the acid solubility of enamel surfaces.[9] Because of this variation it was not possible to obtain sufficient data to correlate fluoride uptake with tooth type, tooth appearance, or age. It is our impression that teeth which appeared to have a rough, porous surface, had a high uptake, possibly because they allowed diffusion of fluoride ions into the enamel. The high counts obtained on buccal compared to lingual surfaces may be related to different degrees of abrasion, because of the greater accessibility of buccal surfaces to toothbrushing. The structural or chemical characteristics of the enamel which make adjacent and similarly appearing areas react differently are unknown.

Because of the increased fluoride uptake found in pumiced compared to intact surfaces it may seem justified to subject teeth to frequent pumicing. However, the rapid loss of fluoride by abrasive action will more than supersede subsequent gain in uptake, and overzealous pumicing will be apt to cause an over-all loss of fluoride from the tooth.

No attempt was made to quantitate the uptake of radioactive fluoride in terms of actual quantities of fluoride. The geometry of the surface counting method employed renders this type of calculation difficult and open to wide errors. While such information would be valuable in determining the mechanism of the fluoride reaction, it was felt that during the preliminary stages of this work, data based upon the relative uptake by various surfaces would be adequate.

SUMMARY

1. A study has been made of the uptake by enamel surfaces of radioactive fluoride from sodium fluoride solutions containing 1 ppm of fluoride. The uptake varies in different teeth and different areas on the same tooth.

2. Brushing with water caused a rapid loss of fluoride during the first 3 minutes and decreasing loss thereafter. Brushing with abrasives removed

most of the fluoride after 30 seconds, demonstrating that there was only a slight fluoride penetration into the enamel.

3. The rate of uptake was greatest during the first few minutes of exposure and decreased with time.

4. Pumiced, ground, or acid-etched surfaces showed greater uptake than intact surfaces.

5. Slightly demineralized areas, e.g., brown or white spots, showed greater uptake than sound enamel.

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THE EFFECT OF TOPICALLY APPLIED POTASSIUM FLUOROSTANNITE ON THE DENTAL CARIES EXPERIENCE IN CHILDREN

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IT HAS been demonstrated by Knutson and Armstrong[1] Galagan and Knutson,[2] Jordan, Wood, Allison, and Irwin,[3] Howell, Gish, Smiley and Muhler,[4] and McLaren and Brown[5] that 4 topical applications of an unbuffered aqueous 2 per cent solution of sodium fluoride results in approximately 30 to 40 per cent less new dental caries in children 1 year after the initial treatment series. However, laboratory[6-10] and clinical[4, 5, 11] studies indicate that stannous fluoride is more effective than sodium fluoride in reducing the incidence of dental caries when applied posteruptively to the teeth of children. Due to the instability of stannous fluoride in solution, present clinical evidence suggests that it must be prepared and used fresh in order to obtain its maximum effectiveness. A new stannous fluoride compound, potassium fluorostannite, appears to have the advantage of increased stability in solution, but lacks clinical investigation. Rat studies indicate that its activity in reducing dental caries is similar to that of stannous fluoride.[12] These studies also have shown that its toxicity is similar to that of stannous fluoride or sodium fluoride. In this paper, clinical data will be presented comparing the effectiveness of 4 topical applications of a 4 per cent solution of potassium fluorostannite to that of a similar number of applications of distilled water.

EXPERIMENTAL PROCEDURES

A total of 894 children, aged 6 through 15 years, from Brazil, Indiana, were initially treated in this study program. The initial age and sex breakdown of this group is seen in Table I. These children were selected by providing the parents of all children in grades 1 through 8 the opportunity to fill out a questionnaire and request that their children be included in the study program. Over 90 per cent of the parents completed the questionnaires and returned signed requests. These questionnaires were screened to eliminate all children who had ever received fluoride treatments or lived in a known fluoride area.

Each child was given a detailed oral examination using mouth mirrors and explorers under artificial light. Portable chairs were used and compressed air was available for use at the discretion of the examiner. No radiographs were used. All examinations were performed by the same dentist (C. W. G.).

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TABLE I
THE AGE AND SEX BREAKDOWN OF THE CHILDREN COMPRISING THE TWO STUDY GROUPS

AGE	CONTROL GROUP		POTASSIUM FLUOROSTANNITE GROUP	
	MALES	FEMALES	MALES	FEMALES
6	13	15	21	16
7	28	50	28	27
8	27	30	25	28
9	20	25	29	15
10	22	19	28	22
11	28	35	27	27
12	19	28	19	22
13	23	35	35	25
14	28	12	19	16
15	3	2	10	2
16 and over	2	0	1	0
Totals	213	251	242	200

The children were divided into 9 susceptibility classes on the basis of previous dental caries experience and dental age. Within each susceptibility class they were then assigned to either Group A (distilled water) or Group B ($KSnF_3$) by a randomizing process which maintained equal numbers of subjects in each of the 2 groups.

Group A received a thorough prophylaxis followed by a series of 4 applications of distilled water. The first application was given immediately following the prophylaxis while the second, third, and fourth applications were given 2 to 7 days apart. The technic described by Knutson[13] was used except that the teeth were kept moist throughout each 4-minute treatment period. Each treatment consisted of isolating one half of the mouth at a time with cotton rolls, thoroughly drying the teeth, and completely wetting each tooth surface with the solution by means of cotton applicators.

Group B received a prophylaxis and 4 applications of a 4 per cent solution of potassium fluorostannite.* The technic of application was identical with that described for the group receiving distilled water. Since this solution was considered more stable than stannous fluoride, fresh solutions were prepared only as needed for mass application. This was usually once or twice a day. After approximately 10 months, the children were re-examined by the same dentist using the same equipment and procedures. A blind examination was performed so that the examiner did not know what treatment each child received or what caries experience was previously recorded.

DATA AND DISCUSSION

At the time of the second examination, 801 of the original 894 children were available for re-examination. There were 411 subjects in the control group and 390 in the experimental group. The dental caries balance at the initial examination is shown in Table II. It is seen from these data that good balance between the 2 study groups was achieved with respect to the previous dental caries experience as evaluated by the DMFT or DMFS indices or the number of noncarious teeth present.

*The potassium fluorostannite was furnished by Dr. William F. Bruce, 900 North Broad Street, Philadelphia, Pa., to whom we are indebted.

TABLE II
THE PREVIOUS DENTAL CARIES EXPERIENCE AT THE TIME OF THE INITIAL EXAMINATION

GROUP	NUMBER	SEX	MEAN DMFT	MEAN DMFS	MEAN NONCARIOUS PERMANENT TEETH
Control	213	Male	5.77	10.28	12.18
	239	Female	6.08	10.66	12.59
$KSnF_3$	242	Male	5.51	9.96	11.52
	200	Female	5.79	10.10	12.79

The dental caries increment in terms of DMFT and DMFS during the one-year period is shown in Table III. The average increment in DMFT was 1.21 for the control group and 0.56 for the potassium fluorostannite group, representing a 53.7 per cent reduction in the dental caries experience in the experimental group. When the total DMFS index was used for comparative purposes, the potassium fluorostannite group showed a 39.2 per cent reduction in comparison to the control group. When the caries increments in terms of the various components of the DMFS index were used to evaluate the effectiveness of the potassium fluorostannite solution, approximately the same degree of caries reductions were shown as seen by the use of the total DMFS index.

TABLE III
A COMPARISON OF CARIES INCREMENTS AFTER 10 MONTHS IN TWO GROUPS OF CHILDREN ONE OF WHICH RECEIVED FOUR APPLICATIONS OF 4% SOLUTION OF POTASSIUM FLUOROSTANNITE AND THE OTHER FOUR APPLICATIONS OF DISTILLED WATER

	CONTROL	EXPERIMENTAL	PER CENT REDUCTION
Number of subjects	411	390	
DMFT increment	1.21	0.56	53.7 ($p = <0.000015$)
DMFS increment	2.42	1.47	39.2 ($p = <0.00014$)
Proximal	1.00	0.73	27.0
Occlusal	0.86	0.44	48.8
Buccal-lingual	0.55	0.30	45.4

The results of this clinical study indicate that potassium fluorostannite has a pronounced anticariogenic effect. It is impossible to translate these findings to a comparison with stannous fluoride since the 2 would need to be evaluated simultaneously in the same experiment. However, the effectiveness of stannous fluoride judged by the one-year data of McLaren and Brown[5] would indicate that the compounds are of the same degree of effectiveness. The final criterion of superiority between any group of fluorides which are to be used topically may well rest on the duration of the effectiveness. Since this study with potassium fluorostannite is being continued, additional data will show whether there is a significant effect after one year. However, it is of interest to point out those 3 tin containing fluoride salts, stannous fluoride, stannous chlorofluoride, and potassium fluorostannite all have pronounced anticariogenic effects. This would suggest that more work is needed in this area in an attempt to find still more effective combinations of fluorine with tin or other similar elements.

CONCLUSION

Ten months following the initial treatment of 442 children with a 4 per cent aqueous solution of potassium fluorostannite, there was a reduction of

dental caries of 53.7 per cent and 39.2 as evaluated by the DMFT and DMFS indices, respectively, when compared to a similar group of 452 children receiving four applications of distilled water. These differences were highly significant.

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A NEW APPROACH TO THE TOPICAL APPLICATION OF FLUORIDES FOR THE REDUCTION OF DENTAL CARIES IN CHILDREN

A Preliminary Report

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THE presently advocated procedure for topical application of fluorides[2] requires 4 visits to the dental office once every 3 years in order to obtain the maximum benefit. As a result many dentists have not adopted the use of topical applications as a routine office procedure. Therefore, in order to develop a procedure which would be simplified, it was decided to compare the anticariogenic effectiveness of a single application of stannous fluoride with that of the conventional four applications of sodium fluoride. Stannous fluoride has been shown to be more effective than sodium fluoride in reducing powdered enamel solubility,[3] rat[4] and hamster[5] dental caries, and human dental caries.[6-8]

A total of 554 second grade children from Montgomery County, Indiana, were used in this study. Only second grade children were used because this program is designed so as to continue throughout the next five years by continuing the treatment each year and by adding new second graders as they enter school. Each of the children was given a detailed oral examination using mouth mirror and explorer under artificial light. Portable chairs were used and compressed air was available for use at the discretion of the examiner. All examinations were performed by the same dentist (C. W. G.).

The children were divided into 9 susceptibility classes on the basis of previous dental caries experience and dental age. Within each susceptibility class they were then assigned to Group I (sodium fluoride) or Group II (stannous fluoride) on the basis of estimated caries expectancy.[9]

Group I received an aqueous unbuffered 2 per cent solution of sodium fluoride (pH 6.5), the first application of which was given immediately following a thorough prophylaxis by the whole mouth technic described by Knutson.[2] The second, third, and fourth treatments were given 2 to 7 days apart. Group II received a prophylaxis and a single application of an aqueous 8 per cent unbuffered solution of stannous fluoride (pH 3.0). The technic of application of the solutions was the same except that the teeth treated with stannous fluoride were kept moist with the solution throughout the 4-minute treatment time. Due to the instability of stannous fluoride in solution, it was prepared fresh every hour in order to avoid chemical changes that might occur in aqueous solution.

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After 8 months,* the children were re-examined by the same dentist using the same equipment and procedures. The examiner did not know what treatment each child received or what caries experience was previously recorded. A total of 489 of the original 554 children were available for re-examination. The balance of the dental caries experience between the groups was good (Table I).

TABLE I

THE EFFECTIVENESS OF ONE APPLICATION OF AN 8 PER CENT SOLUTION OF SnF_2 AS COMPARED WITH FOUR APPLICATIONS OF A 2 PER CENT NaF SOLUTION

	NaF	SnF_2	"SUPERIORITY" (%)
Number of subjects	242	247	
No. of sound permanent teeth at start	8.3	8.1	
DMFS at start	2.9	3.0	
DMFT increment	0.76	0.60	21 (p 0.08)
DMFS increment	1.25	0.93	26 (p = 0.025)
Proximal	0.16	0.14	12
Occlusal	0.70	0.57	19
Buccal-lingual	0.39	0.22	44

In Table I, the dental caries increments in terms of the DMFS and DMFT indices are shown, as well as the components of the DMFS. The average increment in DMFT for the sodium fluoride group was 0.76 while for the stannous fluoride group it was 0.60, or a difference of 21 per cent. Thus, 1 application of the solution of stannous fluoride was 21 per cent more effective in preventing new noncarious teeth from becoming carious than 4 applications of a 2 per cent solution of sodium fluoride. This difference is significant at the 0.08 level of confidence.

Based on the DMFS index, there was a difference of 26 per cent in favor of stannous fluoride. This index represents the total new carious surfaces which occurred during the test, and is probably the best estimate of the effectiveness of the 2 fluorides. The difference of 26 per cent between the 2 groups is significant at the 0.025 level of confidence.

The results of this clinical study indicate a significant advantage of 1 application of an 8 per cent solution of stannous fluoride over the conventional series of 4 applications of sodium fluoride. Even if the ability of the 1 application of stannous fluoride to prevent dental caries were only the same, the reduction in the number of treatments from 4 to 1 would be a worth-while achievement. This study was designed to evaluate the effect of 1 application a year, since it is probably more realistic to believe that more children see their dentist once a year than once every 6 months. One treatment per year is also probably more applicable to school programs.

*It is recognized that 1-year data would be more preferred, but the time interval of 8 months resulted from the fact that a stannous fluoride dentifrice along with other fluoride dentifrices was marked in this area after this study was in progress for 8 months. It was considered important to have the first evaluation of the results of these treatments without the complicating factor of having children use the commercial fluoride dentifrices.

SUMMARY

The application of an 8 per cent solution of stannous fluoride once to the teeth of 247 second grade children resulted in 21 to 26 per cent greater reduction in new caries lesions after 8 months as compared with 242 second grade children treated 4 times with a 2 per cent sodium fluoride solution.

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THE EFFECT OF VANADIUM PENTOXIDE, FLUORIDES, AND TIN COMPOUNDS ON THE DENTAL CARIES EXPERIENCE IN RATS

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THROUGH the use of a variety of experimental conditions, it has been established that stannous fluoride behaves differently from sodium fluoride with respect to its ability to reduce experimental dental caries, both in animals[1, 2] and in human beings.[3-5] However, the exact reasons for its superiority have not been established, although much previous work has indicated that the cation must influence its activity. In an attempt to learn the reason for this compound's apparent advantage in effectiveness over sodium fluoride, it was considered of interest to determine whether the form of the tin compound furnishing the stannous ion might contribute significantly to its superiority. Previous work has indicated that combinations of stannous fluoride with sodium fluoride and of stannous chloride with sodium fluoride were not as effective as stannous fluoride itself.[1, 6] However, since the total fluoride and stannous ion concentrations were low in these experiments, this factor may restrict comparisons in animals to those between stannous fluoride and other combinations which furnish both the stannous and fluoride ions in solution. It is known, however, that the chemical state of the tin compound definitely influences the activity of stannous fluoride, since fresh solutions are more effective than aged solutions.[6]

Geyer[7] has reported that vanadium pentoxide, administered to hamsters in the diet or by subcutaneous injection, inhibited dental caries to an unspecified extent. Hein and Wisotzky[8] found that hamsters receiving 10 ppm of vanadium (as V_2O_5) in the drinking water developed higher caries scores than did their littermate controls. The latter authors felt that, because of the differences in procedure, the results of the 2 studies are not necessarily contradictory. In view of these findings, it seemed interesting to investigate the effect of vanadium pentoxide on caries in the rat by the procedures used in these laboratories of administering the anticariogenic substances in the drinking water. Some consideration of possible toxic manifestations was also indicated, since toxicity was not discussed in the previous reports. Results with 5 water-soluble fluoride compounds are also presented.

PROCEDURE

This experiment was divided into four separate series, since the number of animals involved was so large as to make a single study difficult to conduct

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and control adequately. In the first series, weanling Sprague-Dawley strain rats were divided into 7 groups according to sex and initial body weight. They received supplements of tin and fluoride compounds in the drinking water as shown in Table I.

In the second experiment similar weanling rats were divided into 5 groups according to sex and initial body weight as in series I. The additions to the drinking water of these animals are shown in Table III.

Series III was composed of 5 experimental groups of weanling Sprague-Dawley strain rats, each equally subdivided according to sex and initial body weight. Each group initially contained 50 animals. Group I received 40 μg/ml. of vanadium as vanadium pentoxide, Group 2 20 μg/ml. of vanadium, and Group 3 10 μg/ml. of vanadium. Group IV was composed of rats which received 20 μg/ml. fluoride as ammonium silicofluoride, and Group V served as a control.

In series IV, a total of 250 weanling Sprague-Dawley strain rats were divided as in series I. Groups 1 through 4 received 20 μg fluoride per milliliter as sodium hexafluorostannite, sodium fluoride, stannic fluoride, or potassium fluoride, respectively. Group 5 served as a control.

All of the solutions were prepared fresh once each week, and no special precautions were taken to prevent oxidation or hydrolysis of any of the tin compounds. The extent to which differences in stability of the solutions may have influenced these results is not known. In all instances the compounds were added to the animals' drinking water, which they also received ad libidum. No attempt was made to regulate the volume of water consumed by any of the animals, but visual observations indicated no major differences in water consumption by any of the different groups. All of the animals received the same stock corn cariogenic diet* which contained 0.6 μg/Gm. of fluorine. The animals were housed in pairs in raised screen cages in an air-conditioned room. The duration of the experiments was 140 days, after which time the animals were sacrificed by ether and the heads were examined for dental caries as previously described.[6] Four representative male and female rats from each group were analyzed for total fluorine in the carcass according to procedures previously described.[9] The animals used for fluorine analysis from each group were selected in such a manner that their final weights were all comparable.

DATA AND DISCUSSION

The anticariogenic effects of the various tin compounds used in series I are seen in Table I. Results of statistical analysis (Table II) show that neither stannous gluconate nor stannous chloride caused a significant reduction in caries incidence, as compared with the controls. These observations are not inconsistent with those previously reported,[10] which showed that the stannous ion alone, without fluoride, had only a slight articariogenic effect. The marked effect of fluorine is demonstrated by a significant difference in the dental caries

*Yellow corn grits, 52.7%; ground yellow corn, 11.3%; powdered whole milk, 30.0%; alfalfa, 4.8%; iodized sodium chloride, 1.0%; and irradiated yeast, 0.2%.

TABLE I

A COMPARISON BETWEEN THE DENTAL CARIES EXPERIENCE OF RATS RECEIVING DIFFERENT TIN COMPOUNDS IN THEIR DRINKING WATER (SERIES I)

GROUP NO.	SUPPLEMENT	FLUORINE CONC. (μG/ML.)	TIN CONC. (μG/ML.)	NO. OF RATS	MEAN NO. OF LESIONS	CARIES REDUCTIONS* (%)
1	Stannous gluconate†	—	90	25	7.2	8
2	Stannous gluconate plus fluorine‡	30	90	26	4.5	42
3	Stannous chloride	—	90	29	6.9	12
4	Stannous chloride plus fluorine‡	30	90	33	4.9	37
5	Stannous fluoride§	30	90	30	3.7	53
6	Stannous chloride	—	180	19	6.4	18
7	None	—	—	33	7.8	—

*Based upon number of carious lesions.

†Furnished by the Metal and Thermit Research Laboratory, Rahway, N. J.

‡As sodium fluoride.

§Prepared at Indiana University by Mr. Joseph Davis to whom we are indebted. See J. Am. Chem. Soc. 74: 1604, 1952, for method of preparation.

TABLE II

STATISTICAL ANALYSIS OF SERIES I DATA*

Treatment Groups

1. Stannous gluconate, 90 ppm Sn^{++}
2. Stannous gluconate, 90 ppm Sn^{++}; NaF, 30 ppm F
3. Stanonus chloride, 90 ppm Sn^{++}
4. Stannous chloride, 90 ppm Sn++; NaF, 30 ppm F
5. Stannous fluoride, 30 ppm F and 90 ppm Sn^{++}
6. Stannous chloride, 180 ppm Sn^{++}
7. Control

ALL POSSIBLE PAIRS	TREATMENT DIFFERENCE	SEPARATION OF TREATMENT BY Q TEST† D (.05)‡	D (.01)
1-2	2.78	2.42	2.59
1-3	0.34	2.42	–
1-4	2.27	2.09	–
1-5	3.54	2.42	2.64
1-6	0.71	2.42	–
1-7	–0.55	2.42	–
2-3	–2.44	2.42	2.49
2-4	–0.51	2.42	–
2-5	0.76	2.42	–
2-6	–2.07	2.40	–
2-7	–3.33	2.42	2.84
3-4	1.93	2.01	–
3-5	3.20	2.42	2.84
3-6	0.37	2.42	–
3-7	–0.89	2.42	–
4-5	1.27	2.42	–
4-6	–1.56	2.42	–
4-7	–2.82	2.42	2.84
5-6	–2.83	2.42	2.84
5-7	–4.09	2.42	2.84
6-7	–1.26	2.42	–

*The statistical analysis was performed by Mr. Robert Lehnhoff to whom we are indebted.

†Scheffe, H.: A Method for Judging All Contrasts in the Analysis of Variance, Biometrika 40: 87-104, 1953.

‡If a treatment difference is as large as or larger than the d-value, a significant difference can be said to exist at the probability level associated with the d-value.

experience when 30 ppm F as sodium fluoride is added to the stannous gluconate or stannous chloride solution. While the caries figures were not significantly different for the animals receiving stannous fluoride and for those receiving stannous gluconate or stannous chloride plus sodium fluoride, the trends shown here do not contradict the previously reported observation that mixtures of $SnCl_2$ and NaF are less effective than is SnF_2 in reducing dental caries in rats.[6]

The dental caries experience in the series II animals is seen in Table III.

TABLE III
A COMPARISON BETWEEN THE DENTAL CARIES EXPERIENCE OF RATS RECEIVING DIFFERENT FLUORIDE COMPOUNDS IN THEIR DRINKING WATER (SERIES II)

GROUP NO.	SUPPLEMENT	FLUORINE CONC. (μG/ML.)	TIN CONC. (μG/ML.)	NO. OF RATS	MEAN NO. OF LESIONS	CARIES REDUCTION* (%)
1	Potassium stannous fluoride† (potassium fluorostannite)	30	60	26	5.6	32
2	Stannous fluoride‡	30	90	29	5.0	41
3	Stannous fluoride plus sodium fluoride	30	45	31	7.2	15
4	Stannous fluoride plus potassium fluoride	30	45	28	6.8	19
5	None	—	—	30	8.4	—

*Based upon number of carious lesions.
†Furnished by Dr. William F. Bruce, 900 North Broad Street, Philadelphia, Pa., to whom we are indebted.
‡Prepared at Indiana University by Mr. Gino Bissagio to whom we are indebted.

The effect of potassium stannous fluoride (potassium fluorostannite) is quite similar to that of stannous fluoride with both producing a significant reduction (Table IV) in the dental caries experience. However, mixtures of stannous fluoride with potassium or sodium fluoride at the same fluorine concentration as furnished by stannous fluoride or potassium stannous fluoride are less effective. These data also indicate that there may be inherent anticariogenic properties possessed by stannous fluoride which are not duplicated by mixtures furnishing similar ions in solution. It cannot be stated at this time whether this difference results from the lowered tin concentration or from an inherent difference in anticariogenic properties between the 2 kinds of solutions.

The amount of fluorine stored in the rat from the different fluorides as determined by total carcass analysis in the series I animals is seen in Table V, and the results for series II are in Table VI. While fluoride intake figures were not obtained in this study, there was no gross indication that the groups differed significantly in fluid consumption. The females stored less fluorine than the males, except in the control groups. That the females stored more fluorine in the skeleton than the males when the intake was low (less than 3 μg/Gm.) corroborates previous work.[11] Similarly, there is no apparent relationship between the amount of fluoride stored and the effect of the compound on the dental caries rate. The data do have a tendency to indicate, however, that the presence of the stannous ion as furnished by either stannous fluoride or stannous chloride interferes with the storage of fluorine in the skeleton when compared to similar

concentrations of fluorine in the presence of stannous gluconate. This is in keeping with other experimental studies which indicate that stannous gluconate does not hydrolyze as rapidly as stannous chloride.

TABLE IV
STATISTICAL ANALYSIS OF SERIES II DATA*

Treatment Groups
1. $KSnF_3$; 30 ppm F
2. SnF_2; 30 ppm F
3. SnF_2 + NaF; 30 ppm F
4. SnF_2 + KF; 30 ppm F
5. Control

ALL POSSIBLE PAIRS	TREATMENT DIFFERENCE	SEPARATION OF TREATMENT BY Q TEST	
		D (0.5)	D (.01)
1-2	0.08	1.70	–
1-3	–1.70	1.68	–
1-4	–1.25	1.73	–
1-5	–2.93	1.72	2.02
2-3	–1.78	1.68	–
2-4	–1.33	1.73	–
2-5	–3.01	1.72	2.02
3-4	0.45	1.72	–
3-5	–1.23	1.70	–
4-5	–1.68	1.74	–

*See Table V for footnotes.

TABLE V
TOTAL FLUORINE IN RAT CARCASSES (SERIES I ANIMALS)

GROUP NO.	SUPPLEMENT	TOTAL F (MG.)	
		FEMALES	MALES
1	Stannous gluconate (90 μg Sn/ml.)	0.43 ± 0.11*	0.31 ± 0.04
2	Stannous gluconate plus sodium fluoride	22.17 ± 3.03	19.75 ± 2.95
3	Stannous chloride (90 μg Sn/ml.)	0.51 ± 0.12	0.43 ± 0.07
4	Stannous chloride plus sodium fluoride	16.85 ± 4.59	13.98 ± 3.02
5	Stannous fluoride	18.46 ± 2.00	16.51 ± 1.71
6	Stannous chloride (180 μg Sn/ml.)	0.42 ± 0.08	0.34 ± 0.04
7	None	0.26 ± 0.02	0 31 ± 0.03

*Standard deviation.

TABLE VI
TOTAL FLUORIDE IN RAT CARCASSES (SERIES II ANIMALS)

GROUP NO.	SUPPLEMENT	TOTAL F (MG.)	
		MALES	FEMALES
1	Potassium stannous fluoride	21.9 ± 1.3*	16.8 ± 1.6
2	Stannous fluoride	18.7 ± 3.4	18.0 ± 0.9
3	Sodium fluoride and stannous fluoride	23.5 ± 3.0	17.7 ± 2.3
4	Potassium fluoride and stannous fluoride	20.0 ± 2.5	17.5 ± 2.9
5	None	0.3 ± 0.03	0.32 ± 0.02

*Standard deviation.

The data obtained from the series III animals are found in Table VII. These data indicate that none of the groups of animals which received the solutions of vanadium pentoxide had any reduction in their dental caries incidence. There was a numerical, although not significant, increase in caries in the 2 groups surviving at the end of the experimental period. The group which received the ammonium fluorosilicate had an apparent decrease in dental caries but it also was not significant.

The animals which received the vanadium pentoxide were highly affected toxicologically by the solution. Those which received the highest concentration (40 μg/ml. vanadium) all died within 65 days after first receiving the solution. Some of the animals receiving the less concentrated solution survived the experimental period but in each instance the toxicity of the solution was manifested both by an increase in mortality and by smaller body weight of the survivors.

TABLE VII
THE EFFECT OF VANADIUM PENTOXIDE AND AMMONIUM SILICOFLUORIDE ON THE DENTAL CARIES EXPERIENCE IN RATS (SERIES III)

COMPOUND	VANADIUM CONC. (μG/ML.)	FLUORIDE CONC. (μG/ML.)	SEX	NO. OF RATS AT TERMINATION OF EXPERIMENT	WEIGHT GAIN (GM.)	MEAN NO. OF LESIONS
Vanadium pentoxide	40	–	----------	all	animals	died----------
Vanadium pentoxide	20	–	Male Female	15 12	188 123	9.5 8.9 > 9.2*
Vanadium pentoxide	10	–	Male Female	14 16	212 114	9.0 8.1 > 8.6
Ammonium silicofluoride	–	20	Male Female	20 20	236 148	7.9 7.5 > 7.7
Water	–	–	Male Female	20 19	258 145	8.7 7.9 > 8.3

*Sexes combined.

It is not easy to compare these findings with those of Geyer[7] since he did not mention the effect of the vanadium on the growth of the animals. However, no dental caries reductions were noted in our studies, these findings being similar to those reported by Hein and Wisotzky[8] in the hamster.

In all instances the groups of series IV animals receiving the 4 different fluoride solutions had a directional tendency for less dental caries as compared to the control group (Table VIII). However, only stannic fluoride and sodium fluoride led to significant reductions, but they were significant only at the 0.08 level of confidence. This latter point is of interest since stannous fluoride has been shown to have a highly significant effect on dental caries reduction in both the rat[12-14] and the hamster.[2] Apparently, the tin moiety must be present in the stannous state in order to obtain maximum effectiveness from tin fluoride solutions. These data corroborate previous observations in rats[6] which indicated that freshly prepared solutions decrease the dental caries rate to a greater degree than aged solutions. These findings would also

suggest that solutions of stannous fluoride which are to be used for topical applications should be made and used fresh in order to obtain the maximum effectiveness. However, since the chemistry of tin and fluorine compounds is so complicated, additional studies are needed in this area concerning the effect of oxidation and hydrolysis on the effectiveness of stannous fluoride in inhibiting both human and experimental animal dental caries.

TABLE VIII
A COMPARISON BETWEEN THE DIFFERENT FLUORIDES AND A CONTROL GROUP RECEIVING DISTILLED WATER ON THE INCIDENCE OF DENTAL CARIES (SERIES IV)

	FLUORIDE CONC. (μG/ML.)	SEX	NUMBER OF SURVIVING ANIMALS	WEIGHT GAIN (GM.)	DENTAL CARIES EXPERIENCE		
					MEAN NO. OF LESIONS	EXTENT	MOLARS AFFECTED
Sodium hexafluorostannite	20	Male	21	265	9.0 > 8.5*	1.77	5.54
		Female	20	164	8.0	1.59	5.07
Sodium fluoride	20	Male	18	267	8.1 > 7.7	1.52	5.53
		Female	20	148	7.2	1.37	5.14
Stannic fluoride	20	Male	21	297	8.4 > 7.6	1.73	5.31
		Female	20	152	6.7	1.26	
Potassium fluoride	20	Male	22	271	8.3 > 8.3	1.62	5.22
		Female	21	145	8.3	1.70	5.28
Control	–	Male	17	274	9.9 > 9.6	2.43	5.50
		Female	19	136	9.3	2.07	5.56

*Sexes combined.

SUMMARY

Stannous fluoride (alone and with sodium or potassium fluoride), stannous chloride (alone and with sodium fluoride), stannous gluconate (alone and with sodium fluoride), and potassium fluorostannite were administered to weanling rats in the drinking water for 140 days. Dental caries rates were significantly reduced by stannous fluoride, by potassium fluorostannite, and by stannous chloride and stannous gluconate to which sodium fluoride was added. The stannous salts alone (without fluoride) were essentially without effect. Stannous fluoride supplying fluoride at a level of 30 μg/ml. was more effective than an equimolecular mixture of stannous fluoride with either sodium or potassium fluoride, which furnished fluoride at the same level; in the latter cases the tin level was, of course, reduced by 50 per cent.

The effectiveness of vanadium pentoxide, ammonium silicofluoride, sodium hexafluorostannite, sodium fluoride, stannic fluoride, and potassium fluoride were studied for their anticariogenic effectiveness in rats. None of the solutions significantly reduced the incidence of dental caries except stannic fluoride and sodium fluoride, and these were significant only at a low level of confidence. The vanadium pentoxide solutions were highly toxic as evidence by the failure of the rats to gain weight normally as well as by the high mortality during the study.

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THE GEOGRAPHICAL DISTRIBUTION OF DENTAL CARIES OF YOUNG SCHOOL CHILDREN IN THE NETHERLANDS (PERIOD 1950-1953)

J. B. ZWAARDEMAKER AND S. W. TROMP

Oegstgeest (Leiden), Netherlands

MEDICAL school inspectors in the Netherlands regularly report on the frequency of dental caries among school children. These reports are collected by the office of the Chief Medical Inspector at The Hague. The present data were collected partly by the first author personally in his own districts and are partly based on investigations of his colleagues in other parts of the Netherlands.

For our studies, only the frequency of dental caries among school children of the first class of the primary schools in the Netherlands was taken into consideration. For each school district the percentage of children with perfect dentition was established.

The criteria for "perfect dentition" are rather subjective and not all research workers in the field of dental caries may agree with them. It is very difficult to select purely objective criteria. Depending on the method used (microscopic or macroscopic investigation) the same dentition may be considered "perfect" or "with caries elements." In order to be able to compare the investigations of the various school inspectors, it was decided that a dentition is "perfect" if it has less than 2 caries elements, using macroscopic identification methods, i.e., the caries element can be identified without microscopic enlargement or x-ray analysis.

From most school districts frequency percentages are available for the years 1950, 1951, 1952, and 1953. For the year, 1950, and the average of the 4-year period 1950-1953, a map was prepared indicating the geographical distribution of dental caries among young school children (6 and 7 years of age) in the Netherlands (Figs. 1 and 2).

The average percentage of children with perfect dentition for the Netherlands as a whole in 1950 was 44 per cent; in 1951, 40 per cent; in 1952, 37 per cent; in 1953, 33 per cent.

The maps were colored on the basis of these country averages. School districts with percentage of perfect dentition below the average of the country, in other words, areas with high frequency in dental caries, are called "plus" areas. Those with percentages of perfect dentition above the average of the country, in other words, areas with low frequency in dental caries, are called "minus" areas. Districts in the maps without caries data or insufficient material were left white.

Received for publication Aug. 25, 1956; revised by the authors June 19, 1957.

RESULTS

A comparison of Figs. 1 and 2 indicates a number of interesting features.

1. Despite the general increase in dental caries since 1950, as indicated by the above-mentioned figures, the general geographical distribution does not seem to have changed very much.

2. The province of Zeeland with high-caries frequency borders on the west part of the province of Noord Brabant with low frequencies. The northwest strip of Noord Brabant with very low frequencies is bordering districts with very high frequencies between Rijn-Lek and Maas.

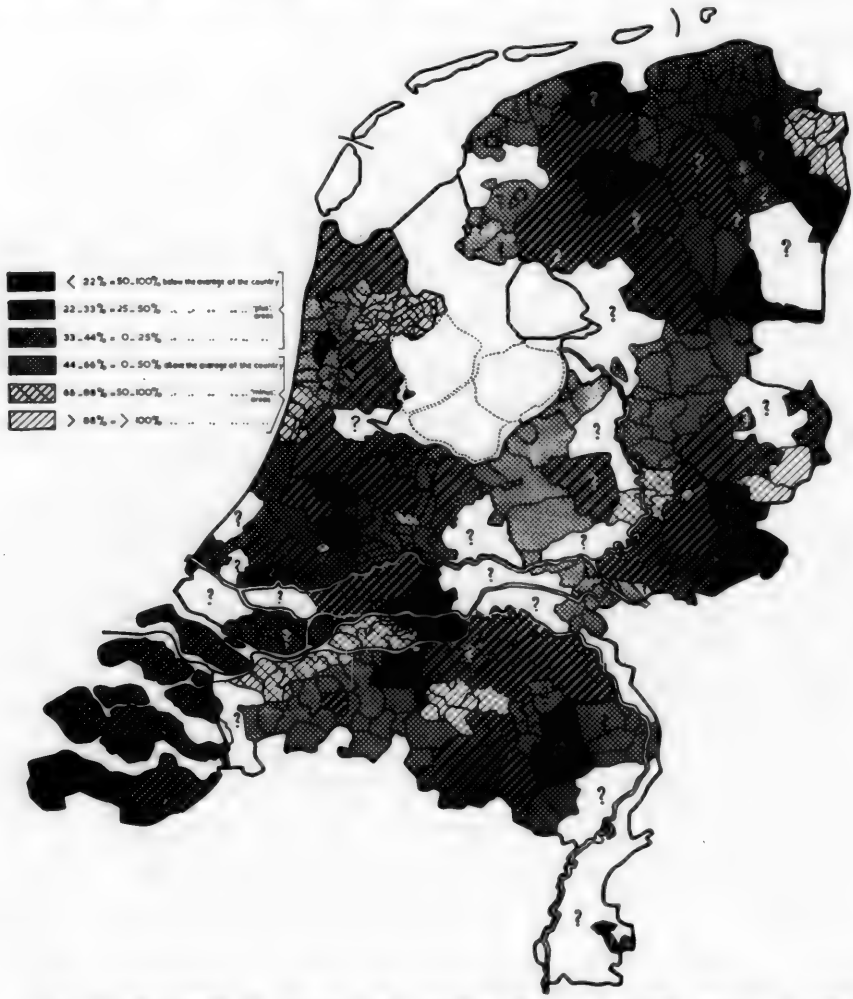

Fig. 1.—Percentages of children with perfect denture per school district; " perfect" means less than 2 caries elements (using macroscopic identification methods). Average percentage of caries frequency in 1950 was 44 per cent; 1951, 40 per cent; 1952, 37 per cent; 1953, 33 per cent. (School districts without caries data remained white. If no data were available for 1950, average values for 1951 and 1952 were used.)

3. a. The "Seaclay areas" of the northern part of the province of Groningen, west part of Friesland, and north part of Noord Holland (West Friesland), in general, are characterized by low-caries frequencies. Zeeland is an exception to this rule.

b. The old "Peat areas" of southeast Friesland, southeast Groningen, Drenthe, and parts of Zuid Holland seem to have high-caries frequencies.

c. The "Sandy areas" of Overijssel, Veluwe, and W. Noord Brabant in general are areas with low-caries frequency.

d. The typical "River-clay areas" seem to have very high-caries frequencies. For an accurate analysis of this soil-caries problem, as has been

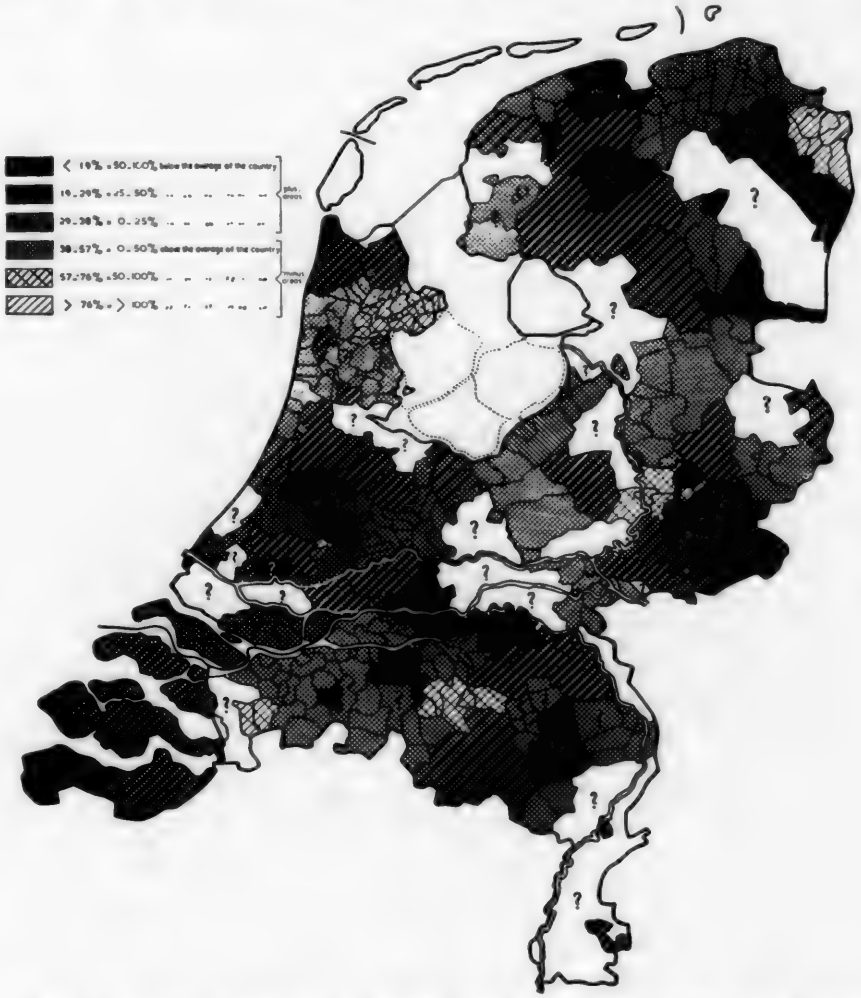

Fig. 2.—Average percentages of children with perfect denture per school district; "perfect" means less than 2 caries elements (using macroscopic identification methods). Average percentage of caries frequency period 1950-1953 was 38 per cent. (School districts without caries data remained white.)

carried out for cancer and other diseases by the second author, caries data per municipality (the smallest administrative unit in the Netherlands) are required.

4. It is evident that pronounced autochthonous population groups often with relatively backward hygienic habits (such as the agricultural population of Zeeland, eastern part of Noord Brabant and De Achterhoek) are characterized by high-caries frequencies.

5. In Table I, for 8 districts of the Haarlemmermeerpolder (a reclaimed inland sea, southwest of Amsterdam) for the years 1947, until 1953, the dental-caries percentages of "perfect dentition" in young school children are indicated. The percentages are based for each year, on approximately 2,500 children. The districts are classified from north to south.

TABLE I

PERCENTAGES OF "PERFECT DENTITIONS" IN 8 DISTRICTS OF A RECLAIMED INLAND SEA, ARRANGED FROM NORTH TO SOUTH

DISTRICT	YEAR							AVERAGE
	1947	1948	1949	1950	1951	1952	1953	
Haarlemmerliede	54.54	83.33	27.27	50.00	33.33	—	30.00	46.40
Halfweg	65.45	73.71	33.80	36.23	26.22	20.00	—	42.56
Badhoevedorp	78.57	52.83	50.70	25.00	25.92	26.31	25.00	40.62
Bennebroek	89.95	46.15	46.66	38.88	—	22.85	15.79	43.38
Hoofddorp	61.36	60.00	34.00	22.03	24.59	7.40	15.91	32.18
Nieuw Vennep	47.05	39.39	—	32.14	31.42	20.00	8.10	29.68
Aalsmeerderbuurt	36.11	—	31.57	28.00	21.05	33.33	17.64	27.95
Abbenes	—	26.66	60.00	12.50	11.53	—	21.42	26.42

— = no data available.

All figures represent percentages of "perfect dentition" in school children of the first class of the primary schools.

It is remarkable that except for the district Bennebroek (which lies at the western outskirt of the Haarlemmermeer), the average percentages of "perfect dentition" decrease from north to south, i.e., the caries frequency increases from north to south.

Further studies are required to explain these geographical differences. It seems that, on the whole, the northern part of the Haarlemmermeerpolder is slightly more urbanized than the south. More people from the north are working in the neighboring cities (Haarlem and Amsterdam). However, the fact that in several typical rural areas of the provinces of Groningen, Friesland, northern part of N. Holland, etc., low-caries frequencies occur (see Figs. 1 and 2) seems to contradict this explanation.

Another possible cause is the difference in soil in the various parts of the Haarlemmermeer. It would be worth while to study whether differences in trace-element content (particularly of fluorine) in soil and water (both ground-water and drinking water) correlate with the above-mentioned decrease in percentages of "perfect dentition."

CONCLUSION

The present study is only a preliminary report. The authors fully realize that the available data are still insufficient to draw any definite conclusions. However, it is felt that detailed medical-geographical studies based on small administrative units may reveal various causes of dental caries which are difficult to find through ordinary clinical studies only.

Erratum

In the paper by N. B. Williams and E. B. Franck (*J. D. Res.* 36: 361, 1957), the term "*L. buchneri* (#11577)" is used (p. 362) instead of the correct name "*L. brevis* (#11577)."

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Annual Meeting—Atlantic City, N. J.

March 21, 22, 23, 1957

EXECUTIVE PROCEEDINGS

(Compiled by Dr. Dan Y. Burrill, Secretary-Treasurer)

EXECUTIVE SESSION 1. March 21, 1957. 8:15 P.M.

Prior to the opening of the business meeting, Dr. Theodore H. Rosebury presented a tribute to Dr. William J. Gies, reviewing Dr. Gies' accomplishments, in particular his service to dental education.

President J. F. Volker called the meeting to order. A quorum was present. Several resolutions were presented concerning Dr. Gies. It was voted that the resolutions be received and that a committee be appointed to prepare a single resolution expressing the sentiment of the entire Association. The president appointed the committee: B. G. Bibby (Chairman), Bion R. East, and Eli H. Siegel.

The minutes of the 1956 Annual Meeting were approved as printed in the Journal of Dental Research for October, 1956.

Tellers (Hampp, Simmons, McCauley, and Dale) were appointed to count the ballots on the amendment of the constitution and by-laws. They reported at the same session a vote of 493 for and 13 against the amendments. The amended constitution and by-laws were declared adopted by affirmative vote of over half of the total membership of the Association. (Membership total—863.)

Tellers (Zander, East, and Kraus) were appointed to count ballots for the election of officers.

The Assistant Secretary-Treasurer presented the financial report of the Secretary-Treasurer's office, with an analysis to the effect that the Association had been operating at a deficit for some years.

H. Trendley Dean reported on the affiliation of this Association with the A.A.A.S. and also encouraged attendance at the 12th Congress of the International Dental Federation in Rome, September 7-14, 1957.

Thomas J. Hill reported for the committee on incorporation.*

The committee on selection of meeting places, Reidar Sognnaes (Chairman), submitted the following schedule: 1958—Detroit, Mich., Statler Hotel; 1959—San Francisco, Calif., Sheraton-Palace Hotel; 1960—Chicago, Ill., Morrison Hotel; 1961—Boston, Mass., Statler Hotel; 1962—probably St. Louis, Mo.; 1963—probably Pittsburgh, Pa.

Editor Robinson reported on the operation of the Journal of Dental Research. After discussion, the Association voted in favor of a referee system, but strongly against any major change in the format of the Journal; against following specifically the pattern of the Journal of Bacteriology, and against abandoning the "Editor's Viewpoint." The sense of the meeting was that the editor and publication committee should have authority to determine policies.

All reports were accepted and approved.

*Since the meeting, the Association has been incorporated as a corporation not for profit under the laws of the State of Illinois.

COUNCIL MEETINGS

1. March 22, 1957. 8:30 A.M.

Present were: Volker, Sognnaes, Williams, Hatton, Burrill, Bevelander, Crawford, Glass, Grossman, T. J. Hill, Kerr, Lefkowitz, Ludwick, Mahler, Orland, H. B. G. Robinson, Shafer, Simon, and W. F. Swanson.

After discussion the council voted to set the 1958 dues for members of the North American Division of the I.A.D.R. at $15.00 of which $7.00 is to pay for the subscription to the JOURNAL and $8.00 is to go into the general fund of the Association. Dues for other members remain the same as before.

H. D. Coy and G. Bevelander were designated members of the Ad Interim Committee of the Council. It was agreed that this action does not set a precedent to the effect that any group must always have representation on the committee.

Dues for 1958 and following years were remitted for M. Klatsky, W. L. Wylie, and L. T. Austin, and any others the Archives Committee discovers to be eligible. It was further voted that dues remission become semi-automatic under part B-2 (B) of the by-laws, the dues being remitted on the request of the member if he is eligible.

2. March 23, 1957. 8:30 A.M.

Present were: Volker, Sognnaes, Williams, Hatton, Burrill, Arnim, Bibby, Coy, Crawford, Dean, Glass, Hall, Jump, Kerr, Knighton, Ludwick, Orland, Pearlman, H. B. G. Robinson, Rosebury, Shafer, and Simon.

On recommendation of a committee appointed to select candidates (Simon, Crawford, and Shafer), the council nominated Ralph Ireland and John Haldi for possible election as councillors at large.

On recommendation of a committee appointed to investigate (Bevelander, Orland, and W. F. Swanson), the council voted in favor of affiliation with the American Institute of Biological Sciences (A.I.B.S.).

F. J. Orland, chairman of the Archives Committee, submitted a written report detailing the progress of the committee in collecting historical and biographical data concerning the Association, its founders, activities, and members.

R. S. Manly, chairman of the Membership Committee, reported for the committee. After discussion, 50 persons were nominated for election to membership. The council directed that its Committee on Membership set February 15, 1958, as the deadline for receipt of nominations to membership for election in March, 1958, and that the Secretary-Treasurer not forward to the Committee nominations postmarked later than that date.

Council actions reported were by unanimous vote.

EXECUTIVE SESSION II. March 23, 1957. 5:00 P.M.

A quorum being present, the session convened as a meeting of the North American Division of the I.A.D.R. President Volker presided. A resolution of tribute to Dr. William J. Gies (p. 658) was adopted unanimously.

S. Wah Leung was nominated and unanimously elected representative to the A.A.A.S.

The president reported the recommendation of the council that the I.A.D.R. become affiliated with the American Institute of Biological Sciences (A.I.B.S.). The members voted to approve the recommendation and seek affiliation.* The cost to the Association will be $100 each year.

There being no further business, the meeting of the North American Division was adjourned and the session immediately reconvened as a general meeting of the Association.

On nomination by F. A. Arnold, Jr., Senator Lister Hill was unanimously elected honorary vice-president of the I.A.D.R. in recognition of "his continuing interest and efforts in behalf of dental research."

After explanation by the president of the need for special action arising out of the transition from the old to the new constitution, the council nominees for election as councillors at large, Ralph Ireland and John Haldi, were unanimously elected.

*The affiliation has since been arranged.

The president reported the action of the council setting the dues for members of the North American Division at $15 for 1958 ($7.00 for the subscription to the JOURNAL and $8.00 for the general fund of the Association). Dues and subscription fee for members outside North America remain as before ($3.00 dues plus $7.00 for the subscription to the JOURNAL if the member wishes to subscribe). Subscription is not required of members outside the North American Division. It is required of North American members.

A budget for the office of the Secretary-Treasurer, previously approved by the Council, was approved as it appears in the supplement to the proceedings.

The Lincoln Bank and Trust Co. of Louisville, Ky., and the State Bank and Trust Co. of Evanston, Ill., were approved as repositories of Association funds.

The Association approved payment of all expenses of the meeting.

Resolutions of appreciation were adopted: to the members of the Local Arrangements Committee; Louis I. Grossman (Chairman), Neal Chilton, Herbert Cobe, W. Crowell, J. M. Wisan, Seymour Oliet, and to the University of Louisville and the print shop there for services in preparing the pre-printed abstracts.

The Secretary-Treasurer read the names of members deceased during the past year. The assembled members stood in silent tribute.

The committee appointed for the purpose (Basil G. Bibby [Chairman], Bion R. East, and Eli H. Siegel) submitted a resolution of tribute to Dr. Gies. This resolution was unanimously adopted and appears at the beginning of these proceedings.

The names of persons nominated to membership by the council were read and, on motion seconded and passed, a unanimous ballot was cast for their election. The list of persons elected appears in the supplement.

The tellers reported their count of the ballots for election of officers. The newly elected officers were installed.

The meeting was adjourned sine die.

The annual banquet on Friday, March 22, 1957, was attended by 266 persons. Dr. E. H. Hatton was honored by a special program giving a résumé of his career in the fields of scientific research and education. Dr. Reidar F. Sognnaes, then president-elect of the Association, gave a talk on "A Precious Heritage; Footnotes on Ivory Art and Dental Science."

The E. H. Hatton Award for 1957 was presented to Robert L. Smith, College of Dentistry, Ohio State University, for his paper on "The Role of Epithelium in the Healing of Extraction Wounds." George D. Sturman of Yale University received Honorable Mention for his paper on "The Effects of Local Vascular Changes in the Teeth."

The Souder Award was presented to Walter S. Crowell who was given this distinguished honor "because of outstanding scientific achievements as a chemist and engineer, and for research, development and standardization of dental materials."

SUPPLEMENT TO PROCEEDINGS

A. FINANCIAL STATEMENT FOR YEAR ENDED DECEMBER 31, 1956

Office of Assistant Secretary-Treasurer—Louisville, Ky.*

Fund balance, January 1, 1956		$ 5,418.51
Receipts:		
Subscriptions	$4,607.51	
Dues	2,067.77	
Registration Fees and Sale of Banquet Tickets	2,446.50	
Miscellaneous	40.00	9,161.78
Total		$14,580.29

*Audit by Norbert F. Elbert, C.P.A., Louisville, Ky.

Disbursements:

Printing and distributing JOURNAL (C. V. Mosby Co.)		$5,037.04	
Mailing Operations, Postage and Printing		442.49	
Gratuity to Asst. Sec.-Treas.			
Year 1955	$ 500.00		
Year 1956	500.00	1,000.00	
Annual Meeting Expenses:			
Jefferson Hotel	$1,090.27		
Printing Programs, Badges, etc.	521.94		
Speaker and Novice Award	200.00		
Cost of Abstracts	274.99		
Projection Services	628.25	2,715.45	
Equipment Purchased		130.77	
Miscellaneous		239.58	9,565.33
Fund balance, December 31, 1956			$ 5,014.96

Bank Reconcilement

Cash on Deposit—Lincoln Bank & Trust Co., Louisville, Ky.	$4,953.63
Add—Deposit in Transit	141.00
Total	5,094.63
Less—Checks Outstanding	79.67
Balance per Books—December 31, 1956	$5,014.96

B. BUDGET FOR 1957-58

Offices of Secretary-Treasurer and Assistant Secretary-Treasurer

Direct Expenses:

Clerical	$250.00	
Postage	150.00	
Supplies	150.00	
Printing	600.00	
Travel and Misc.	50.00	
E. H. Hatton Award	100.00	
Contingency fund	200.00	
Gratuity	500.00	
Total		$2,000.00

Note: The amounts in each of the categories of direct expense are approximate. Transfers from one category to another as necessary are approved.

C. OFFICERS ELECTED FOR 1957-58 YEAR

President	Reidar F. Sognnaes
President-elect	Ned B. Williams
Vice-president	H. B. G. Robinson
Secretary-Treasurer	Dan Y. Burrill
Asst. Secretary-Treasurer	Edward H. Hatton

Number of members voting—339.

D. NEW MEMBERS ELECTED MARCH 23, 1957

NORTH AMERICA—Douglas Allen Atwood, *Boston, Mass.;* Harrison M. Berry, Jr.,* *Philadelphia, Pa.;* David Bixler, *Indianapolis, Ind.;* Philip J. Boyne, *Washington, D. C.;*

Cosmo R. Castaldi, *Edmonton, Canada;* Frederick M. Deuschle, *Cincinnati, O.;* Lionel Gold, *Camden, N. J.;* R. J. Gorlin, *Minneapolis, Minn.;* Gordon E. Green, *Columbus, O.;* Alvin H. Grunewald, *Chicago, Ill.;* Merle L. Hale, *Iowa City, Ia.;* Norman O. Harris, *Randolph Field, Tex.;* Ernest H. Hixon, *Iowa City, Ia.;* Kirk Conklin Hoerman, *Great Lakes, Ill.;* Charles L. Howell, *Indianapolis, Ind.;* Roscoe D. Hughes,* *Richmond, Va.;* George J. Kasai, *Chicago, Ill.;* John W. Kelly,* *Richmond, Va.;* Leon Kraintz,* *Houston, Tex.;* George D. Kudler, *Brooklyn, N. Y.;* John W. Kumpula, *Washington, D. C.;* Milton Levy, *New York, N. Y.;* Harvey William Lyon, *Bethesda, Md.;* Duncan McConnell, *Columbus, O.;* Inga R. Mahler, *Boston, Mass.;* Charles E. Meyers, *Oakland, Calif.;* Melvin L. Moss, *New York, N. Y.;* Seymour Oliet, *Philadelphia, Pa.;* John Osborne, *Chicago, Ill.,* temp. (U. of Birmingham, England); John K. Peterson, *Bismarck, N. D.;* Paul Phillips, *Madison, Wis.;* Mervyn B. Quigley,* *Medford, Mass.;* John J. Sharry, *Birmingham, Ala.;* I. L. Shklair, *Great Lakes, Ill.;* Lyon P. Strean, *West Point, Pa.;* J. C. Thonard, *Rochester, N. Y.;* Robert Van Reen, *Bethesda, Md.;* D. E. Waite, *Iowa City, Ia.;* James H. Wick, *Iowa City, Ia.;* J. M. Wisan, *Philadelphia, Pa.;* Ozro B. Wiswell, *Houston, Tex.;* Carl J. Witkop, *Bethesda, Md.;* Thomas J. Zwemer, *West Allis, Wis.*

Outside North America—Percy Milton Butler, *England;* Fermin Alberto Carranza, Jr., *Argentina;* T. Graham H. Davies,* *England;* Ronald William Fearnhead, *England;* Walter Alwyn Gaunt, *England;* A. C. Kerr, *England;* Alan Robin Ness, *England.*

E. Necrology

William H. Bauer
G. Friel (S. Africa)
F. C. Friesell
William J. Gies
J. P. Lazansky
C. H. Scheu

F. C. Waite

F. Statistics of 1957 Meeting

Members attending	279
Newly elected members attending	19
Non-members attending	310
Guests	48
Total attendance	656

*Acceptance of membership not received. Art. III, Sect. 4 of the constitution states, "Initiation of membership requires delivery to the Secretary-Treasurer of a signed acceptance of election thereto."

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Proceedings of the British Division

April 11th and 12th, 1957

Fifth Annual Meeting

Institute of Dental Surgery, University of London

1. Lipoid Distribution in the Developing Teeth of the Rat.—*N. B. B. Symons, Dental School, University of St. Andrews, Dundee.* Material from 3- to 5-day-old rats was fixed in Baker's formol-calcium; frozen sections were cut and stained with Sudan Black B to demonstrate lipoids. For phospholipids, Baker's acid hematein method, checked by his pyridine extraction test, was employed. Throughout the cytoplasm of ameloblasts and odontoblasts there is a diffuse sudanophilia, the narrow basal zone of the ameloblast shows, however, a heavy concentration. This basal zone has a considerable phospholipid content. In the nuclear and distal zones of the ameloblasts scattered phospholipid material appears, some of a shape to suggest a thin envelope surrounding secretion globules. In the shortened ameloblast associated with enamel maturation the phospholipid material is absent. No phospholipid or sudanophilia was observed in the dentin or in the enamel matrix.

2. Some Histochemical Observations on the Developing Human Tooth Germ.—*A. R. ten Cate, Anatomy Department, London Hospital Medical College, University of London.* Glycogen distribution in the developing human tooth germ (up to the stage where the first increments of enamel have formed) has been studied by the application of the lead tetra-acetate Schiff method to undecalcified sections. Scattered droplets of glycogen occur within the cuboidal cells of the external enamel epithelium. Below the external enamel epithelium, a 2- to 3-cell layer of glycogen rich cells has been identified. With the appearance of blood vessels along the outer margin of the external enamel epithelium, marked changes in glycogen distribution occur. The ameloblasts show a gradual acquisition of intracellular glycogen until a maximum concentration is reached when the cells are at the "organizing" stage. A rapid decline in glycogen concentration follows, with disappearance from the ameloblasts at the onset of amelogenesis. The odontoblasts contained no glycogen. The disappearance of glycogen from the amelobasts is thought to be due to its phosphorolytic degredation, which in turn, provides hexosephosphate esters, utilized in the formation of the P.A.S. positive ground substance of Tome's process. The absence of glycogen from the odontoblasts, which have a similar basic function to perform as the ameloblasts, is correlated with their better vascular supply. Hexosephosphate esters being obtained from the erythrocytes of the blood, the only other known source, there is no need for glycogen accumulation within the odontoblasts.

3. Observations on the Neuro-Histology of Bone.—*T. G. H. Davies and R. W. Fearnhead, Departments of Children's Dentistry and Dental Pathology and Histology, London Hospital Medical College, University of London.* There are few authoritative accounts of the neuro-histology of bone. De Castro (*Trav.*

du Laborat. de Recherch. Biol. de l'Univ. de Madrid 26: 215, 1930) described nerves terminating on or within osteoblasts. Hurrell (*J. Anat.* [*Lond.*] 72: 54, 1937) demonstated silver impregnated fibers, which he regarded as nerves, ending in the bone matrix. Serial 7 μ and 10 μ paraffin sections, and 15 μ celloidin sections of bone from human, mouse, rat, and monkey material were impregnated with silver by a modified Holmes technic, since with this method it is possible to demonstrate nerve endings in close proximity to calcified tissues (Fearnhead and Linder, *J. Anat.* [*Lond.*] 90: 228, 1956). Nerves in the periosteum of bones from young and adult rats were also stained in vivo with methylene blue. In all the sections examined, numerous well-impregnated nerves were identified in the marrow, Haversian canals, and in the periosteum. In the periosteum many nerves ended in regions close to and between the fibers of tendon and muscle insertions. No nerves were found ending in the bone matrix. Although nerves in the periosteum ended close to osteoblasts, none were found actually ending on the cell surface or within it.

4. Developmental Stages in Oral Epithelial Differentiation.—*C. H. Tonge, Department of Anatomy, Medical School, Newcastle-upon-Tyne.* The sequence in which the various parts of the human embryonic oral epithelium differentiate has been studied using serial microscopic sections of material ranging from 5 mm. to 31.4 mm. crown-rump measurement and corresponding to Streeter horizons XIII—XXIII. At 5 mm. a low columnar epithelium lines the visceral arches internally, being thicker laterally than near the midline. Formed earlier, Rathke's pouch and the tubo-tympanic recess are seen. The median thyroid diverticulum is present. At 11 mm. when the embryonic components of the tongue have united and it occupies the bucconasal cavity, it is lined by a basal layer of cuboidal or low columnar cells with large oval nuclei and one or more layers of flattened cells on the surface. Simultaneously, the epithelium lining the junction of the mandibular and maxillary processes has larger basal cells and greater surface proliferation. The formation of the tooth bearing area is traced in successive stages. At 15 mm. submandibular and parotid gland formation is observed. The submandibular bud is an invagination of low columnar cells with large oval nuclei enclosing irregularly arranged cells continuous with the flattened surface cells. The palatal processes, lined on their oral surfaces by a single layer of cuboidal epithelium, are fusing at the 31.4 mm. stage. Epithelial ingrowths near to the line of fusion are seen. At the same time, some irregularity of the epithelium on the dorsal surface of the tongue is noted, although no papillae are present.

5. The Nature of the Epithelial Attachment.—*James H. Scott and W. A. S. Alldritt, Queen's University, Belfast.* There is still some doubt concerning the origin of the epithelium of the epithelial attachment. According to Orban, it consists of the epithelial tissue of the reduced enamel epithelium, while according to Becks it is derived from the gingival epithelium which replaces the enamel epithelium. The problem has been studied in relation to the erupting teeth of rats, kittens, and rabbits. It was found that the gingival epithelium gradually migrates toward the cementoenamel junction on the outer side of the reduced enamel epithelium, which, however, persists so that the epithelial attachment is, in fact, of dual origin. The inner part is derived from the enamel epithelium; the outer part from the gingival epithelium. The stratum corneum of the gingival epithelium lies between the two epithelial layers for part of the extent of the epithelial attachment. It is suggested that it is this tissue which forms the gingival sulcus and is the most vulnerable area of the epithelial attachment.

6. Nerve Plexuses in the Oral Mucosa.—*A. D. Dixon, Department of Anatomy, University of Manchester.* The topography of submucous nerve plexuses, which give origin to sensory and autonomic nerve terminations, has been studied in selected regions of the oral mucosa in a number of animals employing several neurohistologic methods; including methylene blue, silver impregnation, and the Champy-Coujard staining technics. Uniform distribution of the dyestuffs or fixatives was assisted by pretreating the tissues with testicular hyaluronidase. Nerve bundles ascend toward the mucous membrane giving rise to submucous plexuses which vary within wide limits in their fiber arrangement and density from one part of the mouth to another, many of the preparations emphasizing the profuse nature of the nerve supply to certain regions. Characteristically the plexuses found in the hard palate and dorsum of the tongue are in the form of a close network which lies parallel to the surface, whereas those in the cheeks, inferior surface of the tongue, and floor of the mouth form a more open meshwork composed of smaller bundles of nerve fibers. The nature of the constituent fibers of the plexuses can be shown by the Champy-Coujard technic, a microchemical stain for adrenalin-like substances, by which thick myelinated, or sensory, and extremely fine non-myelinated, or autonomic, nerve fibers can be distinguished. This can be confirmed experimentally, degeneration of myelinated and non-myelinated fibers following division of sensory nerves and the cervical sympathetic trunk, respectively. The sensory fibers give origin to specialized nerve terminations whilst the autonomic fibers form perivascular plexuses and a diffuse terminal nervous reticulum in the submucosa.

7. Odontoblastic Capillary Plexus of Rodent Incisors.—*David Adams, Dental School, University of Manchester.* Perfusion of an India ink—sodium citrate mixture—into the common carotid artery of rats and of rabbits demonstrated the odontoblastic capillary system in the continuously erupting incisors. The method showed that the odontoblastic capillary system is supplied by long arterioles which arise obliquely from the main vessels and course through the pulp, in a forward direction, before turning into the odontoblast layer. At the distal or dentinal ends of the odontoblasts they form a rich plexus anastomosing freely with neighboring vessels. The plexus is, therefore, inter-odontoblastic in its position, immediately adjacent to the predentin zone. This arrangement, which is not found in human teeth, is thought to be related to the rapid formation of dentin in the rodent incisors. The study is being continued on golden hamsters and mice.

8. Some Observations on the Optical Properties of the Amelodentinal Junction.—*E. B. Fanibunda, Department of Dental Pathology, Institute of Dental Surgery, University of London.* An examination of transverse ground sections of teeth shows a narrow translucent zone in the enamel, immediately adjoining the amelodentinal junction. This zone was believed to be highly mineralized (Keil, *Dtsch. Zahn-, Mund. u Kieferheilk.* 6: 393, 1939) because it was shown to be strongly double refracting (Cape and Kitchin, *J. A. D. A.* 17: 193, 1930; Keil, *Dtsch. Zahn-, Mund. u Kieferheilk.* 6: 393, 1939). According to Gustafson (*Odont. Tidskr.* 53: 106, 1945), this zone does not differ from other parts of the enamel and the strong double refraction is due to the parallel arrangement of the prisms in this region. It was found that the width of the translucent zone could be varied by altering the direction of transmitted light. Therefore, it was postulated that, depending on the direction and the angle of incidence of illumination, light rays reflected from the amelodentinal junction into the adjoining enamel produced this appearance of a translucent zone. Experiments were carried out by transmitting light at varying angles of incidence

to the amelodentinal junction. It was concluded that the appearance of the translucent zone could partly be accounted for by the light reflected from the amelodentinal junction.

9. Further Studies on the Action of Collagenase on the Organic Matrix of Sound and Carious Human Dentin.—*W. G. Armstrong, Institute of Dental Surgery, University of London.* A quantitative method has been developed for the estimation of collagenase action on dentin. This has confirmed most of the earlier work of Prophet, Evans, and Atkinson (*Lancet,* 258: 290, 1950; *Brit. D. J.* 94: 278, 1953), but has shown that decalcified carious dentin is only partially resistant to the enzyme, being broken down at a slower rate than decalcified dentin derived from sound teeth. An early feature of collagenase attack has been shown to be the release of a peptide containing hydroxy-proline, aspartic acid, glutamic acid, and glycine. An investigation has been made into the report (Konetzka, Burnett, and Pelczar, *Brit. D. J.* 100: 156, 1956) that dentin demineralized at pH 7.3 by ethylene-diamine-tetra-acetic acid (EDTA) is resistant to collagenase; this has failed to show that EDTA-decalcified dentin is any less susceptible to the enzyme attack than the HCl-decalcified substrate. Blocking or removal of reactive groups known to be present in the collagen molecule has been studied in relation to susceptibility to collagenase attack. It has been found that deamidation or N- and O-acetylation, of the organic dentin matrix partially inhibits its breakdown. Reaction of the (-amino groups of the hydroxy-lysine and lysine present in dentin collagen with fluorodinitro-benzene renders the matrix almost completely resistant to collagenase. Conversely blocking, by methylation, of the free carboxyl groups of the aspartyl and glutamyl residues has no inhibitory effect upon the rate of breakdown.

10. A Histologic and Microradiographic Study of the Peri-Tubular Translucent Zone in Dentin.—*G. C. Blake, Department of Pathology, Institute of Dental Surgery, University of London.* By preparing frozen sections of teeth incompletely decalcified with ethylene-diamine-tetra-acetic acid, adjusted to pH 7 with NaOH, it was found possible to obtain decalcified and undecalcified areas in the same section without the usual shrinkage which accompanies normal decalcification and paraffin embedding technics. Sections were obtained in which a comparison of the translucent zone appearance before and after decalcification could be made in the same microscopic field. By staining these sections with Schmorl's picro-thionin it was shown that, after decalcification, no organic matrix remains in the translucent zone and the picro-thionin precipitate fills the whole of the translucent zone area. Microradiographic examination of ground sections, using x-rays of 2 to 5 kv., before and after decalcification, confirmed this finding. Using the mercuric sulphide precipitation technic, previously described (Blake, *J. D. Res.* 35: 961, 1956), it was shown that the diameter of dentinal tubules in interglobular spaces is equal to the diameter of translucent zones in the adjoining calcified areas. Microradiographic examination of interglobular spaces in ground sections confirmed that these areas are completely uncalcified. These findings indicate that the formation of the translucent zone is a process of calcification in a non-collagenous matrix, separate from the dentin collagen matrix calcification, but that the translucent zone calcification does not take place independently of collagen calcification.

11. Fluorescence Microscopy of Healing Gingival Epithelium of Dogs. —*W. D. McHugh* (present address: *Royal Dental Hospital, London*) *and P. A. Persson, Royal Dental School, Malmö, Sweden.* Gingivectomies were performed on the clinically healthy gingivae of young dogs and the apical extent of the

wound was marked by cutting a groove in the root surface. The dogs were sacrificed at intervals of 2 to 76 days after operation and the blocks of tissue removed, embedded, and sectioned. When the tissues were fluorchromed with Congo red and coriphosphine and primulin at pH 5 and examined with ultraviolet light, it was possible to obtain good differentiation between epithelial cells of different ages. The process of healing of the epithelium was thus analyzed and was found to take place in 4 distinct but continuous stages: (1) Up to the second day, cells from the deeper layers of epithelium adjacent to the cut edge find their way into the edge of the clot. There are few or no mitoses. (2) Approximately 2 to 9 days. Active proliferation of basal epithelium some distance from the healing edge of the border of the area of inflammation leads to the formation of large peglike processes which extend into the underlying connective tissue. These appear to act as "factories" for the production of new epithelial cells which stream out from them through the epithelium to the healing edge, where they pass out over the surface. (3) Nine days onward. When the surface of the granulation tissue is covered by 4 or 5 layers of epithelial cells, the cells in the deepest layers commence to divide. Fine rete pegs now appear and the proliferation of the cells in the deeper layers serves to complete the closure of the wound with a layer of epithelium. (4) In some cases, an overgrowth of the rete pegs in the newly formed epithelium is seen. These form long thick branching processes with, in many cases, degeneration of the central cells.

12. Early Matrix Changes in Caries of the Dentin.—*E. W. Bradford, University of St. Andrews, Dental School, Dundee.* The decalcification of early carious lesions of the dentin with chelating agents, embedding in ethylene glycol (Miles and Linder, *J. R. micro. Soc.* 72: 199, 1953) and subsequent sectioning and staining with Gram (Claudius' modification) shows that the material within the dentinal tubules gradually becomes granular and Gram + under the influence of the carious process. The granules which take up the space normally occupied by the translucent area and the dentinal process of the odontoblast are much smaller than bacteria. The possibility of them being invading organisms is also discounted by the gradualness with which the granularity appears, and the gradualness with which the granules themselves become Gram +. As noted by Engel (*J. D. Res.* 27: 681, 1948) these intensely Gram + tubules are recoverable from the floor of a carious cavity, presumably where degeneration of the contents has not been followed by bacterial invasion. This Gram + property of the tissue has in the past been taken as indicative of bacterial invasion, but this is not so. It is merely an enhancement of the normal staining properties of the tissue. The matrix of the translucent areas and, therefore, the plugs of sclerotic dentin normally stain in sections prepared as above with methyl violet and carbol gentian violet, and even in the normal tissue this tends to be the last portion to lose the stain during differentiation of a gram-stained section.

13. Attached Denticles Following Injury to Late Tooth Germs in Man.—*Martin A. Rushton, Guy's Hospital, University of London.* Colyer (Variations and Diseases of the Teeth of Animals, Bale, London, 1936), regarded multiple tusks in elephants as resulting from mechanical injury to persistently growing pulps. Glasstone (*J. Anat. Lond.* 12: 86, 1952) demonstrated that rabbits' molar germs had regulative capacity in culture when divided before the twenty-second day, but not later when odontoblasts and dentin had appeared. A group of specimens suggests that in man considerable regulative capacity is retained even when the crown is half formed. These are lower incisors of the second dentition damaged at 2 to 3 years of age. The dislocated half-formed

crown has been sharply angulated labially and an attached denticle has subsequently formed on the labial side. Lesser degrees of dichotomy are also found in both lower and upper incisors similarly injured, the angulation being toward the palate in the upper jaw. The denticles are regarded as the result of regulation of extruded portions of the dental papilla and odontogenic epithelium.

14. Histophysical Studies of Scorbutus. The Teeth.—*Richard C. Greulich and Ulf Friberg, Department of Medical Physics and Histology, Karolinska Institutet, Stockholm, Sweden.* Both the etiology and pathogenesis of scorbutic lesions have been the subject of a wide variety of experimental investigations. While the morphologic aspects of the disease have been well described, there is still uncertainty regarding the primary biochemical site of action of vitamin C deficiency. The disease seems to cause a failure of specific synthetic processes which normally lead to the formation or renewal of chemical constituents of the connective tissues, especially the mucoproteins. We have undertaken a comprehensive investigation of acute scorbutus in rapidly growing guinea pigs, as compared to normal and pair-fed control animals, and of various stages of recovery from the disease. The methods which have been applied include: (1) autoradiographic and chemical studies of S^{35}-sulfur metabolism; (2) autoradiographic and microradiographic studies of mineral metabolism following administration of Ca^{45}; (3) organic mass distribution studies by ultra-soft microradiography; and (4) histochemical and histologic studies. Preliminary observations indicate that the rate and extent of uptake of mineral calcium and organic sulfur in dentin is reduced markedly in scorbutus. It is slightly reduced in the pair-fed animals. During recovery from scorbutus, the uptake exceeds normal values. The sulfated component of dentin matrix is not chondroitin sulfate. Scorbutus does not affect the incorporation of organic sulfur by enamel matrix. No striking differences of mass distribution in mineral or matrix have been observed thus far.

15. Intermediate Cementum.—*H. J. J. Blackwood, School of Dental Surgery, Royal Dental Hospital, University of London.* The so-called intermediate cementum is characterized by a zone of protoplasmic inclusions between the dentin and cellular cementum of the root. It is present in the apical portions of the roots of most permanent canine, premolar, and molar teeth and it is also formed to some extent in the bifurcation of the roots of molar teeth. The protoplasmic inclusions can be seen in ground sections of the root but are more easily examined in decalcified sections stained by the picro-thionin method. Fine canalicular connections pass from the inclusions to the terminal dentin tubules on one side and to the canaliculae and lacunae of the cellular cementum on the other. The protoplasmic inclusions of the intermediate cementum are thought to be the remains of incompletely differentiated cells occurring along the developing cementodentinal junction. Rapid growth of the apical portion of the root or disturbance of the root during formation are considered to be contributory factors in the formation of the intermediate cementum layer. Histologic evidence supports these theories, and the connections of the intermediate cementum layer have been demonstrated. However, it has not yet been possible to demonstrate the presence of nuclear material within the inclusions.

16. Experiments on the Role of the Periodontal Tissues in the Eruption of the Rabbit Mandibular Incisor.—*A. R. Ness, M.R.C. External Staff, Department of Physiology, University College, London.* The experimental object was to divide the left incisor transversely at its middle and to pin the basal

half to its alveolus, leaving the other half free to erupt. Incisor eruption rate was measured twice a week for 1½ weeks before operation and then for 5½ weeks. In 3 of the 6 experimental rabbits the "crowns" of the operated incisors were shortened by about 4 mm. Sham operations were performed on 3 further animals: the pin was withdrawn and the incisor not completely transected. Contrary to findings in similar experiments on rats (Massler and Schour, *Am. J. Orthodont.* 27: 552, 1941) the eruption rate of the incisor divorced from its growing base was much reduced. The mean rate of operated incisors was 25 μ per day—being least in the shortened incisors—as against 214 μ per day in the controls. The results suggest that the periodontal membrane is not a prime mover in the normal eruption of the rabbit incisor; but it is notable that human teeth in the eruptive phase move at rates around 30 μ per day. That the separated fragment should move at all is of interest in the light of recent studies on the connective tissue elements underlying wound contraction (Abercrombie, Flint, and James, *J. Embryol. exper. Morphol.* 4: 167, 1956).

17. Some Observations on the Jaws and Teeth of an Opodymous Kitten.—*A. Gaunt, Department of Botany and Zoology, Sir John Cass College, London.* An analysis of serial sections of the head of an opodymous kitten shows: (1) The presence of an unpaired median jaw element associated with the left mandibular ramus. (2) A broad tongue base posteriorly generates bifid tongue lobes anteriorly. A median mass of connective and glandular tissue in the tongue base divides, and becomes incorporated in the bifid tongue lobes, the left of which shows the greater development. An anterior accessory tongue lobe is associated with the lower median jaw element. (3) The dental lamina of the upper median and lateral jaws is continuous. The lamina does not extend into the median maxillary region, right or left, and so only the upper median incisor teeth are present. The paired dental lamina of the lower median jaw element is independent of the mandibular lamina. (4) There are 38 deciduous teeth in the jaws, in addition to 2 lower molar teeth, making a total of 40 in all. Each deciduous tooth has its corresponding member of the permanent set, either in the "cap" or "bell" stage of development. In no instance has the process of tooth eruption commenced. (5) Upper and lower lateral teeth have normal crown topography. Upper and lower median teeth have simplified crown topography. Ameloblast, odontoblast, and pulp systems appear quite normal, as also do their products. (6) Evidence generally points to dominance of the left snout.

18. The Teeth of the Echinoidea.—*A. B. MacGregor, D. G. MacInnes, and E. A. Marsland, Dental School, University of Birmingham.* Many attempts have been made to define accurately the meaning which is attached to the word "tooth." There are, however, so many exceptions to the usual forms and functions of teeth that it is virtually impossible to put forward any detailed definition which will satisfactorily include the anomalies, as well as the more normal forms of dentition. Owen was aware of this difficulty, and as a primary definition he stated that "teeth are firm substances attached to the parieties of the beginning of the alimentary canal, adapted for seizing, lacerating, dividing and triturating the food, and are the chief agents in the mechanical part of the digestive function." In addition to the similarity of position and function, the teeth of the Echinoidea resemble those of the vertebrates also in being composed of tissues which appear to be analogous to enamel and dentin. Chemical analysis, however, shows that they are composed largely of calcium carbonate, in the form of calcite, with only a trace of calcium phosphate. This is almost exactly the reverse of the normal vertebrate condition, where the bulk of the calcified tooth is composed of calcium phosphate in the form of

apatite. It is of interest that the soft tissues which appear so similar to those involved in the formation of vertebrate teeth should become impregnated with calcium in a different form. Although the dental appendages of the Echinoidea are often loosely termed "teeth," it is generally considered that true teeth first occur in the primitive forms of fish. From this study, however, it seems that in the circumstances there is some justification for the use of the term. If this be so, it is interesting to find that such highly organized structures occur so far back in the evolutionary scale.

19. Caries in 5-Year-Old London County Council School Children in 1955 Compared With Earlier Years.—*May Mellanby, Hampstead, London.* In 1929, the teeth of some 1,300 5-year-old children attending London County Council (L.C.C.) schools were examined. Structure was poor, very few of the children were caries free, and only 4.7 per cent could be classified as "free or almost free from caries." (According to the standards used, smooth, shiny teeth were of good structure and those showing M- or G-hypoplasia were defective. A child designated as caries free had no history of present or past decay.) In 1943, with the consent of the L.C.C., a comparable survey was made in, as far as possible, the same schools. The improvement was striking; structure was much better and 24.2 per cent of the children were then caries free or almost caries free. Other similar surveys were made in 1945, 1947, 1949, 1951, and 1955, when the percentages in this category were 28.1, 37.5, 24.9, 23.5, and 21.2. The reasons for these variations, especially since 1947, in this 5-year-old age group cannot be stated categorically. It is felt, however, that the improvement from 1929 to 1947 was partly, if not largely, attributable to the better calcifying diets of pregnant and nursing women and infants, particularly during the war years, when special allocations of milk, eggs, and cod-liver oil were made available to them; moreover, the national diet was enriched by the addition of calcium to flour and of vitamins A and D to margarine, while the consumption of carbohydrates in the form of cereals, sugar, and sweets was reduced and more potatoes were eaten.

20. Biometrical Study of Developing Deciduous Dentitions.—*M. V. Stack, M.R.C. External Staff, Dental School, University of Bristol.* Weights (in mg.) of the teeth comprising 30 immature dentitions were found to correlate with dental developmental ages (fetal ages less 16-24 weeks) by formulae of the type $K \log W = \log T$, where K was 0.63 or 0.64 (molars), 0.65 or 0.67 (maxillary incisors), 0.75 (mandibular incisors) and 0.8 (canines). The growth rate of canines appeared to increase about 3 months after birth. During this period the decrease in growth rate of the incisors corresponded to the completion of enamel deposition, dentin growth rates continuing without alteration. Growth rates were related to the sizes of the teeth. In the case of whole dentitions the value for K was above 0.68, when weights were expressed in centigrams. Ages were counted from the twentieth fetal week ($T = 0$) according to calculation and to earlier histologic observation (Gantz, *D. Cosmos* 64: 131, 1922) that incisor tips are visible at the sixteenth week and second molar cusp tips at the twenty-fourth week. Neonatal growth of teeth is delayed ("neonatal line" of Rushton, *D. Record* 53: 170, 1933) and 2 weeks were subtracted from postnatal ages according to the findings of Schour and Massler (*J. D. Res.* 16: 350, 1937). The ratio of the extreme dentition weights (0.1 and 3 Gm.) was so large that the growth rate calculations are not thought to be affected by individual variations in teeth. Coefficients of variation between 5 and 10 per cent were noted for the weights of the various types of teeth, in a series of 20 neonatal dentitions, when such weights were expressed as percentages of the corresponding dentitions weights.

21. THE DISPLACEMENT OF THE MANDIBULAR DISC IN EXPERIMENTAL ANIMALS.—*R. Sprinz, Department of Oral Anatomy, University of Sheffield.* Six-week-old rabbits were operated on and were sacrificed at 4 and 8 weeks. The mandibular disc was dissected free from its posterior attachments to the condyle and joint capsule. The operations were performed unilaterally and bilaterally. On releasing the disc, it slipped anteriorly, the condyle becoming partially denuded of the disc posteriorly. The results of the experiment indicate that: (1) The disc did not become firmly reattached to the capsule and remained displaced anteriorly. (2) The mandibular condyles became enlarged, similar enlargements have been described following meniscectomy (Sprinz, *J. Anat. Lond.* 88: 514, 1954). (3) The displaced disc became attached to either superior or inferior articular surface, its mobility thereby being reduced. (4) The disc became altered microscopically, showing at times cartilage cells and areas of calcification. These results were checked against control operations in which the joints of 6-week-old rabbits were opened and the synovial fluid allowed to escape. The findings of this series indicate that only minor changes in the joint occurred, and these were restricted to the condyle, which showed slight indentations. The disc in a Rhesus monkey was also freed and the result was a perforation of the disc as well as an enlargement of the condyle. The perforation of mandibular discs in man has been presented by Steinhardt (*Deutsch. Zahnheilk Vortr.* 91: 1934), who suggested that the disc was worn away. The evidence of the experiments is that the perforation of the disc does not occur following its displacement in rabbits. The displacement of the disc in a Rhesus monkey did not show such a perforation. The condylar enlargements are not due to the operative procedure, but are related to lesions of the disc.

22. SOME EFFECTS OF A THYROID HORMONE ANALOGUE (TRIAC) ON RAT INCISOR DENTINE.—*P. J. Holloway, W. R. Merrington, and Rosalind Pitt-Rivers, Medical Research Council, London, and University College Hospital Medical School, London.* Weanling rats were thyroidectomized with 330 μC I^{131} and 5 weeks later provided with drinking water containing 0.5 μg TRIAC (tri-iodothyroacetic acid), an analogue of tri-iodothyronine. Others were injected with I^{131} but received no TRIAC. The animals were killed 7 weeks later, by which time some of the incisor teeth had broken. Incisor teeth from both groups were decalcified, sectioned and stained with hematoxylin and eosin. The thyroidectomized rats had normal dentin; those that had received TRIAC in addition showed very abnormal calcification of the dentin. The matrix was poorly calcified and the predentin wide, with an uneven boundary between the dentin and predentin. TRIAC alone given to normal rats at the same dose level also produced defective dentin. It is concluded that thyroidectomy apparently does not affect incisor dentin formation. This has been shown both by destroying the gland with I^{131} and by removing it surgically. In the latter case, the parathyroids were microdissected free of thyroid tissue and re-implanted. Sufficient I^{131} to effect almost total thyroidectomy does not destroy the function of the parathyroid glands, which in the rat are buried in the substance of the thyroid. After such treatment the teeth show no signs of parathyroid deficiency. I^{131} has a direct and transient effect upon dentin formation. TRIAC in some yet unknown way produces faulty calcification.

23. EVIDENCE OF VASOMOTOR ACTIVITY IN HUMAN GINGIVAL CAPILLARY NETWORKS.—*P. H. Staple, M.R.C. External Staff, Dental Unit, University College Hospital Medical School, London.* Very nearly all the superficial capillaries in the labial marginal gingiva contain some erythrocytes, irrespective of whether blood is flowing (Staple, *J. D. Res.* 54: 783, 1955). In some terminal loops this stasis apparently persists for several days, yet subsequently typical streaming

of erythrocytes is resumed. Consequently capillary patterns appear unchanged in face of considerable changes in blood flow. Although terminal capillary loops in which blood flow is observable may momentarily empty of erythrocytes they have not been seen to undergo "active" contraction during this process. If loops be forcibly distended, e.g., by passage of corpuscular aggregates, they normally regain their previous dimensions. Lightly stroking the gingiva can elicit sudden stasis in terminal loops along the path of the instrument, streaming of erythrocytes being resumed ½ to 1½ min. later. Spontaneous vasomotion has been observed in (?) metarterioles (Zweifach, *Anat. Rec.* 73: 475, 1939) running perpendicular to marginal terminal loops. Local heating caused a metarteriole to dilate, but raising body temperature to 99.2° F. had no effect or tended to cause it to constrict. One hot day, a large fraction of the superficial capillary bed in $/\overline{3}$ labial marginal gingiva, normally filled, remained empty of erythrocytes for 15 min. (duration of observations). Presumably this resulted from arteriolar constriction. Attempts to reproduce this reaction in $\underline{1}/$ region in another subject by raising body temperature failed.

24. Sensitivity in Dentin.—*L. V. Howard, Guy's Hospital Dental School, University of London.* Various solutions were applied to freshly exposed dentin in teeth showing early carious lesions. The basis of the choice of solutions rested on the clinical observation that sweet things sometimes cause dental pain and, on experiments of Armstrong et al. (*J. Physiol.* 120: 326, 1953), which showed that bare nerve endings in the skin could be stimulated by various substances including acetylcholine and potassium chloride. The solutions chosen for this investigation were 1M and 2M sucrose, acetylcholine 10^{-2} Gm. per milliliter and 1M potassium chloride. They were applied at 37° C., twice in each subject and in random order. Twenty-four subjects were used, and the cavities were prepared without an anesthetic with slow-running burs kept moist. Out of a total of 48 applications of each solution, 2M sucrose produced 23 painful responses; 1M sucrose, 9; 1M potassium chloride, 6; and acetylcholine, 3 painful responses. If the sensation of pain from dentin depended on the direct stimulation of nerve fibers at the exposed ends of cut dentinal tubes, acetylcholine and potassium chloride would have been expected to cause pain with a greater frequency than was found. The results so far obtained, however, do not provide for a satisfactory explanation for the mechanism of sensitivity in dentin.

25. A Method of Measuring Physiologic Tooth Movements in Man.—*D. C. A. Picton, Department of Special Anatomy, Guy's Hospital Dental School, University of London.* Vertical movements of a tooth under pressure from opposing teeth can be demonstrated and measured by the use of a resistance wire strain-gauge. The strain-gauge is stuck to a spring which is anchored to part of the dental arch, excluding the test tooth. Vertical movements of this tooth, transmitted to the spring by means of a fine wire, distort the spring and therefore bring about a change in the strain-gauge resistance. This resistance change is measured on a Wheatstone bridge circuit and is related to movements of the spring by calibrating outside the mouth using a moving-stage microscope with an eye-piece micrometer.

26. Masticatory Loads.—*D. J. Anderson and D. C. A. Picton, Guy's Hospital Medical and Dental Schools, University of London.* Using an intradental strain-gauge apparatus calibrated to record "whole tooth loads" as previously described (Anderson, *J. D. Res.* 35: 671, 1956), with crustless bread as the test food, masticatory loads were measured in 3 experiments: First, with alternate chewing sequences on the strain-gauge tooth and on the opposite side of the mouth. Second, chewing on the normal strain-gauge tooth and the same tooth

with its occlusal surface raised by approximately 0.02 inches (0.127 mm.). Third, chewing on the normal strain-gauge tooth and on the same tooth with part of its occlusal surface including the strain-gauge unit lowered by approximately 0.02 inches (0.127 mm.). Four subjects were used and they chewed a total of 20 pieces of bread in each experiment. In the first experiment, the load pattern showed a remarkable similarity on the chewing and nonchewing sides of the mouth, suggesting some evenness of load distribution over the dental arch. In the second experiment, although the recording tooth was the only one to meet the opposing arch and therefore might be expected to take heavy loads, there was frequently little difference between the normal and "raised-bite" loads. In one subject the "raised-bite" loads were approximately twice the normal. In the third experiment the only means whereby loads could be transmitted to the lowered recording surface was through the intervening layer of food. In this series, the load pattern differed considerably from the normal, and measurable loads were only recorded in the early part of each chewing sequence.

27. The Organic Components of Human Dental Enamel.—*K. Little, Nuffield Orthopaedic Centre, Oxford.* The electron microscope may play an important part in the early stages of a systematic study of enamel caries, that is, in finding out exactly what enamel is made of and what happens to it as caries develops, but technics need to be suitably modified. It is essential to remove the embedding medium. Interpretation is greatly helped by viewing photographs of sections stereoscopically. Using the electron microscope, it has been shown that the organic matrix of developing enamel has 2 components. The denser seems to be a form of keratin, and the less dense the compound described by Stack (*J. A. D. A.* 48: 297, 1954) as "soluble protein." In the very early stages of development, the keratin is compact, later it becomes more open, so that both components form a spongelike network. The less dense compound forms a continuous phase, with islands of keratin present arranged in a prismlike manner, but with few complete "prisms." There is evidence that crystallites are laid down in the less dense compound before they are laid down in the keratin. In chalky enamel, either from early caries or following the Manley and Brain treatment (*Brit. D. J.* 87: 199, 1949), the first component to dissolve is the soluble protein, followed much more slowy by the crystallites embedded in it. The keratin protects the remainder of the crystallites.

28. Fluoride Exchange at the Tooth Surface.—*J. L. Hardwick and J. H. Fremlin, Department of Dental Surgery, University of Birmingham.* Fluoride absorption at the tooth surface, following immersion in or rinsing with 1.0 ppm solution of fluoride as the sodium salt, labeled with F^{18}, has been studied by autoradiographs and Geiger counting. After such applications followed by prolonged rinsing or washing with tap water containing 0.05 ppm in fluoride, considerable concentrations of the applied fluoride remain on or near the enamel surface. The degree of concentration of the applied fluoride varies greatly between different teeth and over the same tooth; the highest concentrations are usually found on the cementum, at or near the amelocemental junction, on deposits of calculus, on areas where the surface layers of the enamel have been removed by stoning or where the enamel has previously been exposed to the action of a sodium lactate-lactic acid buffer (pH4.5), and in parts of early carious lesions. Small quantities (under 1.0 mg.) of sodium lactate-lactic acid buffer (pH 4.5) may contain between 0.1 and 7.0 ppm of the applied fluoride after remaining in contact under conditions which prevent evaporation for periods between 5 and 30 minutes with enamel surfaces which had previously been immersed in the 1.0 ppm fluoride solution and then washed in tap water.

29. THE EFFECT OF FLUORINE ON HARD TISSUES.—*S. M. Weidmann and J. A. Weatherell, Biological Research Unit, School of Dentistry, University of Leeds.* Differences in the chemical composition of cortical and adjacent exostosed bone from fluorosed sheep, cattle, and rabbits have been investigated. The main differences between the exostosed and the adjacent cortical bone are that in the former there is an apparent increase in the Ca:N ratio and a decrease in the Ca:CO_2 ratio, while the Ca:P ratio remains normal. The results suggest that exostosed bone is incompletely calcified, and the reduction of the CO_2 content of this bone may be due to replacement of the CO_2 radical by fluorine ion. Significant increase in the serum alkaline phosphatase activity was observed in fluorosed animals. Histologic studies show a proliferation of osteogenic cells, metaplasia of the osteoblasts, increased osteoclasis, the production of woven bone, and its eventual replacement by lamellar bone. The latter point was confirmed by polarized light microscopy. Cartilage and osteoid seams were demonstrated in the osteophytic bone by metachromatic staining, the presence of osteoid seams being confirmed by silver staining of a nondecalcified section.

30. FLUORIDE UPTAKE IN VIVO IN ENAMEL DEFECTS AND ITS SIGNIFICANCE.—*C. M. Dowse and G. N. Jenkins, Department of Physiology, King's College, Newcastle-upon-Tyne.* Myers et al. (*J. D. Res.* 31: 743, 1952) and Hein et al. (*J. D. Res.* 33: 661, 1954) applied F^{18} to teeth and showed that fluoride tends to accumulate in enamel defects including early carious cavities. It seemed of interest to find out whether a similar accumulation of fluoride occurred in such areas in vivo from the fluoride of food and drink. Enamel was ground out from normal and defective areas and was analyzed for fluoride. The results showed that the fluoride concentration in defective enamel was much higher than in sound enamel. This difference was found in teeth from "high fluoride" and "low fluoride" towns but the absolute figures were higher in the former. Any effects which bound fluoride may have (for example, on enamel solubility) are therefore, likely to be greater in defective areas of enamel than elsewhere on the tooth surface. Studies on the solubility of the inorganic matter of enamel by Brudevold's window method have shown that defective parts of the enamel are almost invariably much less soluble than is the intact surface. Results so far indicate that in teeth from "high fluoride" towns, this difference in solubility is greater than in control teeth, i.e., one of the actions of water-borne fluoride may be to depress the solubility of enamel defects.

31. THE pH'S OF DIFFERENT AREAS OF THE MOUTH BEFORE AND AFTER MEALS.—*G. N. Jenkins and I. Kleinberg, Department of Physiology, King's College, Newcastle-upon-Tyne.* The pH's of smooth and interproximal areas of the teeth from all regions of the mouth have been studied, by means of antimony micro-electrodes, before and at intervals after carbohydrate meals. Before meals the differences in pH between mandibular and the corresponding maxillary areas were slight. Within 1 to 2 hours after taking carbohydrate, the pH of the mandibular areas exceeded that of the corresponding area in the maxilla, and lingual areas had higher pH's than labial, with interproximal areas intermediate. Mandibular areas, labial interproximal and lingual, and maxillary lingual areas decrease in pH in an anteroposterior direction. In contrast, the pH's of the maxillary labial and interproximal areas vary in the opposite way, i.e., lower pH's are found in the anterior areas. The role of the various salivary glands in producing these differences can be explained on the basis of their relative rates of flow.

32. TETRACALCIUM MONOHYDROGEN TRIPHOSPHATE TRIHYDRATE; A CONSTITUENT OF DENTAL CALCULUS.—*S. L. Rowles, M.R.C. Staff, Department of*

Dental Pathology, University of Birmingham: present address, *Royal Veterinary and Agricultural College, Copenhagen, Denmark.* The presence of tetracalcium monohydrogen triphosphate trihydrate (the Bjerrum $2^2/3{,}3$ phosphate, also called octacalcium phosphate) noted by Tovborg Jensen, Danø, and Pedersen represents the first detected occurrence of this compound in nature. A series of dental calculus samples derived from the London area have been examined using the Guinier monochromator x-ray diffraction camera, a technic particularly suited to the identification of this compound. The $2^2/3$ phosphate was present in every sample examined, in some being the principal constituent.

33. Free Amino Acids and Phosphoethanolamine in the Salivary Secretions of Man.—*A. C. Kerr, Department of Dental Medicine, Guy's Hospital, London, and G. A. Rose, the Medical Unit, University College Hospital, London.* Samples of parotid gland secretion from 4 normal volunteers, a sample of submandibular gland secretion and a sample of whole saliva also from normal volunteers were ultrafiltered, desalted and examined by 2-way paper chromatography and electrophoresis for ninhydrin reacting substances. The ninhydrin reacting substance present in highest concentration in normal parotid and submandibular secretions was found by confirmatory experiments to be phosphoethanolamine. Normal whole saliva contained considerably less phosphoethanolamine than the individual secretions. A substance was found in it which was absent from parotid and submandibular secretions. This was probably γ-amino butyric acid but may have been δ-amino valeric acid. Parotid secretion from 7 patients with metabolic bone disease, 1 with cystinuria and 1 with polycythemia vera were treated in the same way. The chromatograms did not differ in any striking way from those of the normals. The cystinuric patient showed no excess of cystine, lysine, arginine or ornithine in his parotid secretion. This suggests that there is no mechanism of specific reabsorption of amino acids from the parotid comparable with the renal tubular reabsorption of amino acids. In 2 patients with osteogenesis imperfecta, the concentration of phosphoethanolamine in the parotid secretion was lower than normal. The hypothesis that the concentration of phosphoethanolamine would inversely reflect the plasma alkaline phosphatase concentration was not supported by observations on the parotid secretions of 4 patients with abnormal plasma alkaline phosphatase concentrations.

READ BY TITLE

34. Hereditable Incisor Malocclusion in the Rabbit.—*A. R. Ness, Medical Research Council, Department of Physiology, University College, London.* The abnormality is expressed as protrusion of the lower incisors relative to the uppers, varying in severity from an edge-to-edge bite to absence of any contact with consequent monstrous elongation of the continuously erupting incisors. Histories of animals from different sources show the condition to be hereditable and to occur without relation to sex or body color. Available evidence shows malocclusion starting to develop about 20 days after birth, and that quite serious malocclusion may regress spontaneously to normal. Present studies commence with a litter of 4 abnormal animals: mating of a buck and doe from this has yielded 2 litters, in which malocclusion has developed in 6 of the 12 offspring. Serial radiographs of these litters have shown that 2 features characteristic of the abnormality, opening of the bite and increase in the radius of curvature of the lower incisors, develop only after malocclusion has become established. Outcrossing of another buck of the parent litter to 5 unrelated normal does has yielded 33 normal offspring.

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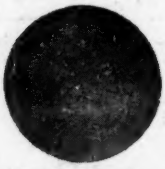

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